ANNUAL RESEARCH PROGRESS REPORT
FY 2001
Grand Forks Human Nutrition Research Center

ANNUAL RESEARCH PROGRESS REPORT

(FY 2001)

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHERN PLAINS AREA

GRAND FORKS, NORTH DAKOTA 58202

MINERAL NUTRIENT REQUIREMENTS

MANAGEMENT UNIT

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0403096 Year: 01 Project Number: 5450-51000-024-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.4 100% %
National Program(s): 107 100%

Title: RELATIONSHIP BETWEEN MINERAL NUTRITION AND RISK FACTORS FOR CANCER

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

It is believed that diet is the single greatest contributor to human cancer, possibly accounting for 35 to 45 percent of the disease. A better understanding of the mechanism and the optimal amounts of trace minerals for cancer prevention can help decrease the burden of cancer in the United States. One possible dietary factor that may increase the susceptibility to colon cancer is inadequate dietary copper. Recent studies have shown that ingestion of a diet deficient in copper significantly increased the formation of carcinogen induced aberrant crypt foci in rats. Aberrant crypt foci are preneoplastic lesions that have been detected in human colon resections and in experimental animals treated with chemical carcinogens. Thus, low dietary copper may be a potential risk factor for colon cancer in humans. In addition, epidemiologic studies have shown that selenium supplementation decreased the risk of and mortality from cancer at a number of sites. A better understanding of the mechanism for the protective effect of selenium, the form of selenium which is most efficacious, and the optimal amount of selenium for cancer prevention is necessary to define human requirements for optimal health and well-being. Studies are and will be conducted to determine the mechanism for the protective effects of dietary selenium and copper against colon cancer susceptibility. Mice genetically susceptible to intestinal cancer will be used to study the effects of trace minerals on the pathogenesis of intestinal cancer in a genetic model for cancer susceptibility. These mice contain a mutation in the murine homolog of the human APC gene and develop spontaneous tumors throughout the intestine. The effects of changes in mineral status on susceptibility of humans to colon cancer will be investigated by feeding people different diets and analyzing the fecal water fraction for cytotoxicity, genotoxicity and alkaline phosphatase activity.

2. How serious is the problem? Why does it matter?

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National Program(s): 107 100%

Cancer is the second leading cause of death in the United States. It has been estimated that the cost for the treatment and care of this disease exceeds $100 billion per year. In addition to the economic impact, the development of cancer may prevent many from enjoying life to its fullest. It is believed that diet is the single greatest contributor to human cancer, possibly accounting for 35-45% of the disease. Dietary excesses, deficiencies and imbalances in trace mineral intake are factors that can affect cancer susceptibility. Thus, providing information about requirements and factors that affect those requirements of mineral elements should result in policies and programs that improve intakes of these nutrients that will result in a healthier population, decrease the burden of chronic disease, enhance the quality of life, and diminish health care expenditures.

3. How does it relate to the National Program(s) and National Program Component(s)?

This research is related to National Program 107, Human Nutrition Requirements, Food Composition and Intake (100%). The research is related to the specific National Program Component of Relationships Between Diet, Genetics and Lifestyle and the Risk for Chronic Disease.

4. What were the most significant accomplishments this past year?

A. Familial adenomatous polyposis (FAP) is a genetic disease useful in the study of colon cancer mechanisms; mutant mice are available that resemble this human condition. These mice that were fed selenium-enriched broccoli had a significantly lower small intestine tumor incidence and a significantly lower small intestine tumor burden than animals fed adequate dietary selenium as selenite. These findings suggest that inadequate selenium-enriched broccoli can decrease tumor development in a genetic model for human cancer.

B. Several observations implicate a role for altered DNA methylation in cancer pathogenesis: the global level of DNA methylation is generally lower and DNA methyltransferase activity is usually higher in tumor cells than in normal cells. Studies were conducted to determine whether a DNA methyltransferase inhibitor would alter the effect of dietary selenium on the formation of aberrant crypts, a preneoplastic lesion for colon cancer. Animals fed a selenium-deficient diet had a significantly higher number of aberrant crypts than animals fed adequate dietary selenium; however, when animals

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National Program(s): 107 100%

were injected with a DNA methyltransferase inhibitor, there was a significant reduction in aberrant crypt formation and dietary selenium did not affect aberrant crypt formation. These results suggest that decreased DNA methyltransferase activity may protect selenium deficient animals against colon cancer susceptibility.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

The frequency of carcinogen induced preneoplastic (precancerous) lesions (aberrant crypt foci) associated with colon cancer development was significantly increased in animals fed low dietary copper and tended to be increased in animals fed low dietary manganese and high dietary iron. Altered activities of antioxidant enzymes, known as superoxide dismutases, were significantly correlated with the number of the anatomical lesions associated with colon cancer. These findings suggest that dietary alterations which affect superoxide dismutase activity affect cancer susceptibility. Furthermore, the effect of dietary copper and manganese of aberrant crypt foci formation may have practical implications because diets in the United States often contain copper and manganese in amounts less than their estimated safe and adequate daily dietary intakes.

Demonstrated that low dietary copper increased the formation of preneoplastic lesions for colon cancer and decreased protein kinase C expression, a series of proteins involved in the signal transduction pathway within the cell. These results provide a biochemical explanation for the increased incidence of precancerous lesions in colons of copper-deficient rats when they are challenged with a carcinogen.

Demonstrated that the amount and the chemical form of selenium in the diet can influence colon cancer susceptibility. Supplementation of a selenium deficient diet with either selenite or selenate but not selenomethionine caused a significant reduction in the amount of carcinogen-DNA adducts in the colon but not in the liver of rats. The formation of carcinogen-DNA adducts is necessary for the first step in cancer development. The reduction in carcinogen-DNA adduct formation in the colon correlated with a reduction in aberrant crypt foci when animals were fed selenite or selenate but not selenomethionine.

6. What do you expect to accomplish, year by year, over the next 3 years?

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National Program(s): 107 100%

In FY 2002, sample collection from a human study to determine the effects of low dietary copper on susceptibility of humans to colon cancer will be completed. Subjects have been collecting their feces and the fecal water fraction has been being isolated. Analysis of the cytotoxicity, free radical production and alkaline phosphatase activity in the fecal water fraction will be performed. These assays will be used as indicators of cancer susceptibility in the whole individual. In vitro experiments will be conducted to determine whether changes in media trace minerals will affect the proliferation, differentiation, DNA methylation and tumorigenicity of human colon cancer cells. Animal studies will be used to determine the mechanism for the protective effects of dietary selenium and copper against colon cancer susceptibility.

In FY 2003, a human supplementation study will be conducted to assess the protective effects of different food forms of selenium on colon cancer susceptibility. Analysis of the fecal water fraction will be used as an indicator of colon cancer susceptibility in the whole individual. In vitro experiments will be conducted to determine the effects of different food forms of selenium in different cancer cell lines (colon, mammary and prostate).

In FY 2004, analysis of the human supplementation study conducted in FY2003 will be completed.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Information about the effect of trace minerals on cancer susceptibility as it becomes available is routinely transferred to a variety of customers. The customers include the public through web pages of professional organizations and via the popular media, and other scientists through presentations at national meetings and professional publications.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

An article, "Nutritionally speaking, infants are not small adults" published in the Grand Forks Herald, July, 2001 and placed on the web site of the Grand Forks Human Nutrition

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Research Center.
An article, "Copper keeps cancer away" published in Self Magazine, June, 2001.
An article, "Broccoli and Colon cancer" published in AICR Science News, December, 2000.
An article, "Chances of colon cancer connected to copper consumption" published in Food and Nutrition Research Briefs, April, 2001.
Interviews on Voice of America and USDA radio.
Articles in the Grand Forks Herald and UND student paper on October 25, 2000 and February 8, 2001.

9. Scientific Publications:

01. Davis, C.D., Newman, S. Inadequate dietary copper increases tumorigenesis in the Min mouse. Cancer Letters. 2000. v. 159. p. 57-62.

02. Davis, C.D., Uthus, E.O., Finley, J.W. Dietary selenium and arsenic affect DNA methylation in vitro in Caco-2 cells and in vivo in rat liver and colon. Journal of Nutrition. 2000. v. 130. p. 2903-2909.

03. Davis, C.D. Diet and Carcinogenesis. Watson, R.R. editor. CRC Press, Boca Raton, FL. Vegetables, Fruits and Herbs in Health Promotion. 2001. p. 273-291.

04. Davis, C.D., Uthus, E.O. Reply to Craig A. Cooney. Journal of Nutrition. 2001. v. 131. p. 1872.

05. Davis, C.D., Hintze, K.J., Whanger, P.D., Finley, J.W. Protective effects of selenium-enriched broccoli against colonic aberrant crypt formation. FASEB Journal. 2001. V. 15. Abstract. p. A62.

06. Davis, C.D., Uthus, E.O. Dietary selenium and methylation status affect dimethylhydrazine-induced aberrant crypt formation in rat colon. FASEB Journal. 2001. v. 15. Abstract. p. A952.

07. Uthus, E.O., Davis, C.D., Yokoi, K. Selenium deprivation decreases the activity of liver betaine homocysteine methyltransferase in rats. FASEB Journal. 2001. v. 15. Abstract. p. A969.

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National Program(s): 107 100%

Publications: (Continued)

08. Nielsen, F.H., Davis, C.D., Milne, D.B. Low dietary zinc and copper negatively affect plasma and urine indicators of bone health. Proceedings of the North Dakota Academy of Science. 2001. v. 55, p. 65.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404367 Year: 01 Project Number: 5450-51000-026-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Title: EFFECTS OF COPPER DEPLETION ON CARDIOVASCULAR FUNCTION AND METABOLISM

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Diets in the U.S. frequently are low in copper in comparison to dietary standards set by the Food and Nutrition Board of the National Academy of Sciences; these diets resemble the low copper diets that have produced abnormal electrocardiograms, increased cholesterol in blood, impaired metabolism of sugar, and poor control of blood pressure in men and women in controlled experiments. Diets low in copper may contribute to human illness (See Question 2).

The approach to the problem is to identify new biochemistry and physiology of copper with animal experiments to provide functional biomarkers useful in dietary experiments with human volunteers and in community studies. The experiments will identify mechanisms by which adequate dietary copper produces beneficial effects, will identify new effects of human diets low in copper and will contribute to the establishment of national dietary standards. People with biomarkers suggestive of low copper intakes will be supplemented and their responses evaluated.

2. How serious is the problem? Why does it matter?

The major signs of copper deficiency found in depleted men and women and deficient animals resemble the most common characteristics that can predict risk of ischemic heart disease in people. Nearly 80 anatomical, chemical and physiological similarities between animals deficient in copper and people with ischemic heart disease have been identified. It seems likely that the low copper diet common in the U.S. contributes to this disease which is the leading cause of death in the U.S., 480,000 deaths annually. The cost of medical care for this illness is more than $5 billion per year which does not include effects of sorrow, time lost from work or annual cost of prevention (at least $1000 per person). Proper selection of foods may yield diets that meet the

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National Program(s): 107 100%

standards necessary to both prevent illness and decrease expense. This work is relevant to dietitians, food companies, physicians, producers of grain, legumes, nuts and other foods high in copper, public health planners, and teachers of nutrition.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research program fits into the National Program 107, Human Nutrition and Performance Goal 3.1.1. Human nutrition requirements. Emphasis is on biomarkers, mechanisms of action, nutrient interactions and functions as related to healthy hearts and blood vessels to optimize longevity, to decrease disease incidence and to improve productivity. Cooperative studies are in progress with Loma Linda University and the Health Research and Studies Center to evaluate the effects of diets high in complex foods on biomarkers of trace element status and with the Medical College of Ohio to clarify the mechanisms by which copper deficiency alters electrocardiograms and heart function.

4. What were the most significant accomplishments this past year?

None, new CRIS.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

None, new CRIS.

6. What do you expect to accomplish, year by year, over the next 3 years?

During FY 2002, the ability of phytochemicals in wine to improve copper utilization by rats will be evaluated because it generally is assumed that it is wine that protects French people from their high fat diet. Experiments will be based on those published a few years ago using beer, but commercially available pure chemicals and wine extracts will be used.

Genetic typing will be used to evaluate data from a human study of the effects of zinc on copper requirements. Several candidate genes have been identified, the most promising of which controls a zinc enzyme related to both heart disease mortality and physical fitness. Zinc intakes are known to greatly affect copper utilization. Another candidate gene that controls the ability to metabolize alcohol with a zinc enzyme

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National Program(s): 107 100%

will be the next gene studied. Other genes are being sought so that they can be evaluated in 2003. A pilot study will be designed using genetic typing so that genetic effects on measurements of trace element status can be sought in appropriate target populations in FY 2004.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

None, new CRIS.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

9. Scientific Publications:

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0403544 Year: 01 Project Number: 5450-51000-026-02 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Title: DETERMINATION OF A NO EFFECT LEVEL FOR COPPER IN DRINKING WATER FOR FUTURE DIETARY SUPPLEMENT STUDY

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Diets in the U.S. frequently are low in copper in comparison to dietary standards set by the Food and Nutrition Board of the National Academy of Sciences. This is of concern because low dietary intakes of copper have been associated with the incidence of some chronic diseases such as ischemic heart disease and osteoporosis. Drinking water can sometimes contribute to total copper intake, but this contribution may be limited by regulatory groups attempting to assure quality potable drinking water, in part, by setting low concentration standards for copper.

Human volunteers will be used to identify the chemical forms and doses of copper most useful in dietary supplementation trials and to measure the concentrations of copper in drinking water intolerable to healthy adults.

2. How serious is the problem? Why does it matter?

Some nutritional scientists believe that the best method of defining the nutritional requirement for copper is to conduct dietary supplementation trials. The chemical form of copper in multinutrient supplements recently has been found to be unabsorbable. Supplements with improved absorbability may cause undesirable acute effects such as transient nausea. Moreover, environmental regulatory agencies are reevaluating the quality of drinking water and may decrease the amount of copper because of these undesirable effects that will mandate a change in water purification processes. This research will help establish the safe and tolerable dose of copper as an absorbable supplement and in drinking water. This work is relevant to the Food and Drug Administration, the Environmental Protection Agency, nutritionists, physicians, researchers on heart disease and osteoporosis and various local and state agencies that must respond to federal standards on water quality.

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Accession: 0403544 Year: 01 Project Number: 5450-51000-026-02 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

3. How does it relate to the National Program(s) and National Program Component(s)?

The research fits into the National Program 107 Human Nutrition and the Performance Goal 3.1.1. Human nutrition requirements. Emphasis is on biomarkers, mechanisms of action and environmental factors.

4. What were the most significant accomplishments this past year?

The tolerable concentration of copper in drinking water is unknown. Men and women drank mineral water varying in copper sulfate content and answered questionnaires about symptoms and responses at the Grand Forks Human Nutrition Research Center and at the Universities of Ulster in Northern Ireland and Santiago in Chile, and in Shanghai China. The copper threshold for transient nausea is approximately 5 mg/1. Because this concentration exceeds the U.S. regulatory level of 1.3 mg/1, the Environmental Protection Agency need not change the regulations.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

A similar project done last year revealed the threshold for copper in distilled water to be 4 mg/1; the data have been accepted for publication.

6. What do you expect to accomplish, year by year, over the next 3 years?

During FY 2002 we will evaluate our data on the response to different amounts of copper sulfate in mineral water and submit it for publication. We will evaluate the usefulness of these data for providing nutritional supplements to people of low copper status. In 2003 we will publish the data and terminate the project.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

No technology transfer.

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Accession: 0403544 Year: 01 Project Number: 5450-51000-026-02 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

9. Scientific Publications:

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404250 Year: 01 Project Number: 5450-51000-028-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: BIOAVAILABILITY OF TRACE ELEMENTS, ESPECIALLY IRON FROM FOOD, & ITS INFLUENCE ON NUTRITURE & FUNCTION

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Concerns about the adverse effects of iron deficiency on cognition and physical performance as well as concerns about high iron stores and the risk of chronic diseases (such as cancer and heart disease) emphasize the need to determine how our ability to absorb and use the iron in food (dietary iron bioavailability) should affect dietary advice for the public.

Both short-term iron absorption and longer-term iron status are being measured in humans consuming controlled diets for several weeks to help determine the true importance of dietary iron bioavailability, and the related impact on practical dietary choices such as consuming less meat, more beans and whole grains, or more tea. The bioavailability of other mineral nutrients, such as calcium, copper, and zinc, may also be affected by such dietary choices, and information on bioavailability of these other mineral nutrients can often be efficiently derived from the same human studies. The iron research will determine the practical importance of dietary iron bioavailability, and how extensively biological adaptation modifies it.

2. How serious is the problem? Why does it matter?

Iron nutrition is a double-edged sword. While adequate iron nutriture in infants, children, and childbearing age women is essential for cognitive development and work capacity, excessive body iron in men and postmenopausal women may increase oxidative stress and the risk of chronic diseases such as heart disease and cancer. Research on dietary iron bioavailability provides information for policy decisions about recommended dietary allowances, food enrichment and fortification standards, and dietary guidelines for the public.

3. How does it relate to the National Program(s) and National Program Component(s)?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404250 Year: 01 Project Number: 5450-51000-028-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

National Program 107, Human Nutrition Requirements, Food Composition, and Intake (100%).

This research is directly related to Human Nutrition Performance Goal 3.1.1 Human Nutrition Requirements and 3.1.3 Nutritious Plant and Animal Products, concerning the priority objective: Bioavailability of Nutrients and Food Components.

4. What were the most significant accomplishments this past year?

The discovery of the specific genetic mutation associated with the iron storage disorder hemochromatosis, and the relatively high frequency of heterozygous carriers among those with of Northern European ancestry, has raised concerns about increased iron absorption and the risk of chronic disease in people with this genotype. We determined the occurrence of this mutation in 103 men and women who had participated in our studies of heme and nonheme iron absorption. Our limited observations with 5 subjects identified as heterozygous for the Hfe C282Y mutation suggested no difference in either heme or nonheme iron absorption, compared with absorption by those with no mutation. If our planned additional observations are similar, these findings suggesting that people heterozygous for hemochromatosis do not have excessive iron absorption will provide evidence useful in making policy decisions about iron fortification of the food supply.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

This project is a continuation of #5450-51000-021-00D, which, in brief, demonstrated that US men partially adapt to differences in dietary iron bioavailability, but premenopausal women adapt considerably less, appearing to meet their higher iron requirements by taking full advantage of the 4-5 greater amount of iron available from a diet that contains lean meat and foods rich in vitamin C, without excessive amounts of phytic acid from legumes, whole grains, and tea. Both men and women, including women with low iron stores, adapted by reducing their iron absorption from food after daily iron supplementation with ferrous sulfate. Human adaptation of iron absorption depended on the form of iron in food: absorption of nonheme iron, the major form of iron in foods, accounted for nearly all of the adaptation, with minimal change in absorption of heme iron, the well-absorbed form accounting for 40% of the iron in meat, poultry and fish. Adaptation was accompanied by changes in fecal ferritin excretion but without changes in serum ferritin.

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Accession: 0404250 Year: 01 Project Number: 5450-51000-028-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

The project continued here also demonstrated reduced iron and zinc absorption, but greater copper absorption from a lacto-ovo-vegetarian, as compared with an omnivorous diet. In addition, we found that a high meat diet did not adversely affect calcium absorption and subsequent retention, or other measures of bone health in post-menopausal women.

6. What do you expect to accomplish, year by year, over the next 3 years?

Year 1: Detailed dietary assessment data on 300 women will determine whether dietary variables (such as meat) that affect short-term dietary bioavailability can be related to long-term iron stores. We will test a new experimental approach to determining zinc requirements in humans, by determining how people adapt their zinc absorption to different dietary zinc intakes.

Year 2: We will determine whether people with the common genetic mutation associated with the iron storage disorder, hemochromatosis, absorb more iron than those without the mutation. We will determine the bioavailability of elemental iron powders commercially produced to fortify cereal grains.

Year 3: We will measure iron excretion rates for women, based on isotopic dilution over a 3-year period, and will relate iron excretion to body iron stores. We will test the usefulness of different chemical forms of iron supplements, especially heme iron, in increasing body iron stores without producing gastrointestinal oxidative damage.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Janet R. Hunt participated as a member of the NAS Institute of Medicine's Standing Subcommittee on Interpretation and Use of Dietary Reference Intakes.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

JR Hunt gave a presentation on "Dietary Reference Intakes" University of North Dakota Dietetic Seminar, Grand Forks, ND, May 3, 2001.

9. Scientific Publications:

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401621 Year: 01 Project Number: 5450-51000-028-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: WHOLE BODY COUNTING AND RADIOTRACER METHODS IN RESEARCH ON MINERAL REQUIREMENTS IN HUMAN NUTRITION

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

To make dietary recommendations and evaluate dietary practices that promote good mineral nutrition for the population, there must be sensitive methods for measuring mineral nutrient absorption, excretion, retention, and food bioavailability. The use of isotopic tracer methodology can effectively contribute to meeting these needs. Specifically, use of a whole body scintillation counter can safely and sensitively determine whole body retention of mineral elements that have gamma-emitting isotopes with short to moderate half-lives, such as cadmium, calcium, copper, iron, magnesium, manganese, and zinc. The whole body counting approach has the advantage of determining mineral retention without volunteer inconvenience, high variability, and incomplete sample collections associated with collecting mineral excretion data. It allows the use of a true "tracer" that does not alter the absolute mass of the mineral under investigation, and is easily and sensitively measured with minimal labor. This agreement provides the expertise of a certified health physicist to cooperate with nutrition scientists at the Grand Forks Human Nutrition Research Center, providing an interdisciplinary approach to answering nutrition questions with whole body counting methodology.

2. How serious is the problem? Why does it matter?

Nutrient bioavailability addresses our ability to effectively utilize the nutrients in food for body biological functions. Two diets that contain similar amounts of a nutrient, such as iron, can differ by as much as 10-fold in the amount of iron nutrient that is absorbed, retained, and utilized by the body. Internationally, zinc deficiency has been observed in humans whose diets contained adequate quantities of zinc, but that zinc was not available because of phytic acid from whole grains or legumes that interfered with

ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0401621 Year: 01 Project Number: 5450-51000-028-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

absorption and lack of protein that enhances absorption. Domestically, there is concern that dietary trends (and some recommendations) to increase whole grains and legumes while reducing animal products in the diet may compromise copper, iron and zinc nutrition. The promotion of mineral supplements in the US may lead to imbalances that affect the absorption and retention of other minerals. To complement new knowledge of the importance of nutrients for optimal health, we must know the bioavailability of and interactions among nutrients from common diets, in order to provide dietary advice to the public.

3. How does it relate to the National Program(s) and National Program Component(s)?

National Program 107, Human Nutrition (100%).
This research is directly related to ARS National Human Nutrition Performance Goals 3.1.1, Human Nutrition Requirements, and 3.1.3, Nutritious Plant and Animal Products, concerning the primary objective: Bioavailability of Nutrients and Food Components.
Question 4D:
This report serves to document research conducted under Specific Cooperative Agreement #58-5450-8-11. Additional details of the research can be found in the reports for the parent CRIS projects 5450-51000-021-00D and 5450-51000-028-00D. Accomplishments this year included health physicist support of projects to assess bioavailability of calcium, iron, and zinc using Ca-47, and Fe-59 tracers in human absorption studies. A mathematical model was developed for geometric corrections related to individual body sizes, using an isotope-specific "broad-beam" planar radiation source, the Antropomorphic Uniform Isotope Source (AUNIS). A "golden standard", consisting of a cylindrical NaI(Tl) gamma ray detector housed in a hermetically sealed lead chamber was fabricated, to monitor background radiation in the Whole Body Counter. These accomplishments support the sensitive detection of gamma-emitting isotopic tracers, providing uniquely sensitive measurements of mineral retention in humans, as affected by nutritional status, dietary sources of nutrients, and the human genotype.

4. What were the most significant accomplishments this past year?

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401621 Year: 01 Project Number: 5450-51000-028-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

See parent CRIS projects 5450-51000-021-00D and 5450-51000-028-00D.

6. What do you expect to accomplish, year by year, over the next 3 years?

See parent CRIS project 5450-51000-028-00D.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

See parent CRIS projects 5450-51000-021-00D and 5450-51000-028-00D.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Along with his Physics REU student, Glenn Lykken presented a poster entitled "Making a bone crusher out of a bone breaker" at the North Dakota Science, Engineering and Mathematics 9th Annual Poster Session Program, North Dakota State University, Fargo, ND, July 25, 2001.

Dr. Lykken gave a presentation entitled "Another look at environmental radon" at MICROS 2001, 13th Symposium on Microdosimetry, Stresa (Lago Maggiore), Italy.

9. Scientific Publications:

01. Lykken, G., Hustoft, J., Ziegler, B., Momcilovic, B. Clean Galena, Contaminated Lead and Soft Errors in Memory Chips. Journal of Electronic Matter. 2000. v. 29. p. 1290-1293.

02. Momcilovic B., Alkhatib, H., Duerre, J., Cooley, M., Long, W., Harris, T., Lykken, G. Environmental Lead-210 and Bismuth-210 Accrue Selectively in the Brain Proteins in Alzheimer Disease and Brain Lipids in Parkinson Disease. Alzheimer Disease and Associated Disorders. 2001. No. 15. p. 106-115.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404559 Year: 01 Project Number: 5450-51000-029-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: HOMEOSTASIS AND BIOAVAILABILITY OF TRACE ELEMENTS IN HUMANS AND ANIMALS

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:
1. What major problem or issue is being resolved and how are you resolving it?

The focus of our laboratory is determination of the health effects of consumption of dietary manganese and selenium. Both nutrients are essential, but they also are toxic at higher intakes. Our research is attempting to define the optimal intakes of these nutrients that result in maximal health benefits but do not result in toxicity.
A. Manganese is an essential element, but its practical nutritional importance has not been ascertained, nor has a safe upper limit of intake been established. Knowledge of safe intakes is needed because of recent speculation that high dietary intakes could have detrimental effects on brain function and behavior. Whether manganese ingested through the diet results in manganese accumulation and predisposition to toxicity, and factors that affect these processes need to be determined.

Human volunteers and animals will be used to determine factors affecting manganese absorption and retention in the body and body tissues. Interactions that may affect manganese metabolism include interactions with other trace elements, food and non-food components of plants and fat composition of animals. Studies will determine whether interactions with iron may affect both iron and manganese uptake and retention. Studies with magnesium deficient animals will determine whether diets high in manganese result in increased risk of magnesium-deficiency heart disease.
B. Supplemental selenium is being consumed by many as a prophylaxis against certain cancers, and for its antioxidant potential. These benefits of selenium depend on the chemical form consumed and different foods have different amounts and chemical forms of selenium. Our laboratory is determining the optimal forms and amounts of food forms of selenium for persons desiring to consume supplemental selenium. We are taking an integrated approach and are examining how selenium incorporates into the food, how food preparation affects that selenium, the basic metabolism of selenium from these foods (determined in laboratory animals) and how humans respond to

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404559 Year: 01 Project Number: 5450-51000-029-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

supplementation with these foods.

The efficacy of food sources of selenium for cancer prevention will be determined in rats injected with a carcinogen and fed diets containing selenium supplied as high-selenium meat, wheat or broccoli. To determine the ability of high-selenium foods to induce selenoprotein synthesis, animals and cultured cells will be supplied radioactive selenium incorporated into meat, wheat or broccoli. Proteins will be chemically separated and examined for the presence of radioactive selenium. The health benefits of these foods to humans will be determined in human supplementation studies conducted in selenium-adequate and selenium-deficient areas.

2. How serious is the problem? Why does it matter?

Studies of manganese intake and retention are important because excessive intakes of manganese result in toxicity symptoms that resemble Parkinson's disease. Recently, it has been suggested that the amounts of manganese consumed through food may be harmful and that efforts should be made to remove it from the food supply. There are little data to support these assertions, but likewise there are little data to refute them. This research will be of value to physicians, health professionals and regulatory professionals who will make decisions regarding the safety and adequacy of manganese in the North American diet.

The studies of food forms of selenium are important because selenium supplementation is a nutritional issue. Most nutrition professionals agree that the best way to consume nutrients is through the food supply, but presently there are no guidelines regarding the best food forms of selenium. Demonstrating that selenium in a food can produce health benefits similar to dietary supplements will benefit the consumer, as well as producers who may use the information to increase the marketability and/or profitability of their product. Studies of food forms of selenium are also important because such studies could lead to the production of specialty crops and/or food products that are sold for their enhanced selenium content.

3. How does it relate to the National Program(s) and National Program Component(s)?

These studies relate to the National Program 107, Human Nutrition. This work is related to specific objectives of Bioavailability of Nutrients and Food Components, Health Promoting Properties of Plant and Animal Foods and to Nutrient

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404559 Year: 01 Project Number: 5450-51000-029-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Requirements.

4. What were the most significant accomplishments this past year?

A. Concerns about the possibility of either manganese toxicity or deficiency in free-living humans consuming mixed western diets, and the paucity of well-controlled human studies addressing these questions, were the impetus for a comprehensive human study that examined health effects of consuming diets made as low or high in manganese as practically possible. Human volunteers were fed less than 1 or 20 milligrams of manganese a day for sixty days, and clinical, neurological and physiological variables were closely monitored. This study was initiated a year previous but has only been completed in the past year. Manganese fed at the maximum or lowest amount practical in a mixed Western diet affected manganese retention but did not have any deleterious health consequences to healthy young women. The results indicate, that in the absence of exacerbating circumstances and/or dietary interactions, there is no practical reason to fear for human manganese deficiency or toxicity.

Studies with animals have demonstrated that high manganese combined with a deficiency of magnesium results in increased risk of heart disease, and that this risk may be mediated by manganese substitution for magnesium in magnesium dependent proteins.

B. Because of the reports of the anti-cancer properties of selenium, many persons would like to consume more selenium through their diet, but different foods contain different forms of selenium, and the optimal food form(s) of selenium have not been identified. We have identified farming methods that result in the production of high-selenium wheat, broccoli and beef. We have determined geographical and geochemical factors that affect accumulation of selenium in these foods and have determined the relative effectiveness of these foods for preventing colon cancer. Determined the relative effectiveness of selenium from these foods for incorporation into selenium proteins.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Demonstrated that supplemental selenium improves the mood of healthy young men. This effect was seen in selenium-adequate and selenium-deficient men. This research adds to the

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Accession: 0404559 Year: 01 Project Number: 5450-51000-029-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

increasing number of studies showing that supplemental intakes of selenium improve health and well-being.

Demonstrated in rats that selenium from high-selenium broccoli was retained and distributed differently than selenium from salts such as selenite and selenate. The difference in the distribution of selenium allows selenium from broccoli to be less toxic to rats than other forms of selenium, and also to have greater anti-cancer activity. This research demonstrates that all selenium-containing foods are not equal, and that health professionals need to consider the source of selenium before recommending increased intakes.

Developed a process to label broccoli with stable isotopes of selenium, and demonstrated in healthy young men that selenium from broccoli did not accumulate to the extent that selenium from salts did. This study demonstrated in humans that selenium from broccoli is less toxic than inorganic chemical forms of selenium.

Demonstrated that selenium from high-selenium broccoli was more effective than salt or amino acid forms of selenium for prevention of colon cancer. Found that high-selenium broccoli sprouts were as efficacious as broccoli florets for prevention of colon cancer.

Described, by using cultured cells, how the cell accumulates manganese and demonstrated that manganese is quickly moved in a direction that results in excretion into the gut, whereas movement in the direction of absorption is slow. This finding shows that animals have developed complex mechanisms to keep manganese from accumulating in the body, and that in the absence of exacerbating factors and interactions, manganese toxicity is probably not of practical concern.

Demonstrated in humans that very little manganese is absorbed, and that absorption is strongly associated with the iron status of an individual. This finding demonstrates that interactions of other nutrients with manganese may potentiate manganese absorption. Thus, future studies of possible manganese toxicity should concentrate on possible adverse interactions.

Demonstrated that magnesium-deficient pigs that are fed moderately high amounts of manganese are at an increased risk of sudden death by heart disease. This finding also demonstrates that interactions of other nutrients with manganese may potentiate manganese absorption/retention, and that studies of possible manganese toxicity should concentrate on adverse interactions.

6. What do you expect to accomplish, year by year, over the next 3 years?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404559 Year: 01 Project Number: 5450-51000-029-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Year 1. The effect of high-manganese low magnesium diets on heart ultrastructure and protein function will be determined. Electron microscopy will be used to determine whether mitochondria structure and function are altered. Electrocardiograms (EKGs) will determine whether the heart rhythm is altered.

The effects of other nutrients and food components on the activity of thioredoxin reductase, a selenium protein that may be important in cancer prevention, will be studied in a prostate-derived cell line.

Will continue to study farming systems that result in the accumulation of selenium in wheat, meat and broccoli, and will develop processing methods that do not alter the biological activity of selenium in the foods.

Year 2. After careful examination of the results of animal studies, a human study will be set up to examine possible adverse interactions between low magnesium and high manganese. Subjects will have extensive medical supervision and heart rate and rhythm will also be extensively monitored.

Animal studies will be conducted to determine the bioavailability of manganese from mutant peas that accumulate abnormally high amounts of manganese. If the peas prove suitable as a source of manganese, further studies will determine whether vegetarian diets are at risk of providing too much dietary manganese.

Farming systems that result in production of high-selenium wheat, meat and broccoli will be studied a second year to determine year-to-year variability in selenium accumulation.

Basic nutritional studies will continue to determine the mechanism of cancer prevention by food forms of selenium.

Year 3: Human selenium supplementation studies conducted in selenium-adequate and selenium-deficient areas will determine the benefits of supplemental selenium for immune function, psychological well-being, antioxidant status and selenium protein activity.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Research was transferred to customers by a variety of mechanisms including:

Human manganese studies were used to formulate manganese Dietary Reference Intakes released by the National Academy of Science.

Presented information regarding the anti-cancer effects of

ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0404559 Year: 01 Project Number: 5450-51000-029-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

selenium from broccoli at the International Selenium Symposium in Venice, Italy.

A Cooperative Research and Development Agreement was developed with General Mills to assist in the development and human testing of high-selenium wheat products.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Technology has been transferred through a variety of activities by writing articles for the local newspaper as follows: Do you sell a commodity or food? Grand Forks Herald, 7/26/00.

9. Scientific Publications:

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0402749 Year: 01 Project Number: 5450-51000-029-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: HIGH SELENIUM BEEF PRODUCED IN NORTH DAKOTA

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

2. How serious is the problem? Why does it matter?

3. How does it relate to the National Program(s) and National Program Component(s)?

4. What were the most significant accomplishments this past year?

This report serves to document research conducted under a trust agreement between ARS and the North Dakota Beef Commission. Additional details of research can be found in the report of the parent project 5450-510000-020-00D.

This project was established to determine if cattle fed a ration with high concentrations of selenium would accumulate selenium in the edible portions of beef. Cattle were obtained from a low-selenium and a high-selenium area of the country and they were fed rations formulated with hay and wheat. One half of the animals were fed low-selenium hay and wheat, the other half were fed high-selenium hay and wheat. Cattle obtained from a high-selenium area and fed a high selenium diet ended up with the highest concentration of selenium in beef. The concentration of selenium in those animals was more than 10-fold the national average, and a modest 100g portion of such meat could provide an optimal amount of supplemental selenium to people. Animals from a high-selenium background accumulated less selenium in the liver and kidneys than animals from a low-selenium background, we conclude that they had developed means of reducing their total body burden.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0402749 Year: 01 Project Number: 5450-51000-029-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

6. What do you expect to accomplish, year by year, over the next 3 years?

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

9. Scientific Publications:

01. Hintze, K.J., Lardy, G.P., Marchello, M.J., Finley, J.W. Areas with high concentrations of selenium in the soil and forage produce beef with enhanced concentrations of selenium. Journal of Agricultural and Food Chemistry. 2001. v. 49. p. 1062-1067.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0402795 Year: 01 Project Number: 5450-51000-029-04 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 100% %
National Program(s): 107 100%

Title: HEALTH BENEFITS OF FOOD FORMS OF SELENIUM

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

2. How serious is the problem? Why does it matter?

3. How does it relate to the National Program(s) and National Program Component(s)?

4. What were the most significant accomplishments this past year?

This report serves to document research conducted under a Cooperative Research and Development Agreement between ARS and General Mills, Inc. Additional details of research can be found in the report of the parent project 5450-510000-020-00D.

Wheat is a primary source of selenium, but the amount of selenium in wheat varies. Wheat purchased from Central South Dakota may contain selenium concentrations 250-fold greater than the national average. Selenium from such wheat was converted by General Mills to a whole-wheat cereal product and then used to formulate rat diets. When rats were injected with a carcinogen, consumption of high-selenium wheat depressed the incidence of colon cancer. If high-selenium wheat is proven effective to prevent cancer, then it may be used to supplement conventional cereal products or it may be used to produce specialty food products.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

6. What do you expect to accomplish, year by year, over the next 3 years?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0402795 Year: 01 Project Number: 5450-51000-029-04 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 100% %
National Program(s): 107 100%

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

9. Scientific Publications:

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0403407 Year: 01 Project Number: 5450-51000-029-05 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: HIGH SELENIUM MEAT, WHEAT, AND BROCCOLI: A MARKETABLE ASSET?

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

2. How serious is the problem? Why does it matter?

3. How does it relate to the National Program(s) and National Program Component(s)?

4. What were the most significant accomplishments this past year?

This report serves to document research conducted under a trust agreement between ARS and USDA/CREES, Initiative for Future Agriculture and Food Systems. Additional details of research can be found in the report of the parent project 5450-510000-020-00D.

Many areas of the Mid-Western USA have very high concentrations of selenium in the soil. In the past, these high-selenium areas have been a liability to agriculture, resulting in toxicity to livestock and potential toxicity to people living in the area. However, many people today are aware of the cancer-suppressive properties of selenium and are seeking to increase their intakes of selenium. This project seeks to take advantage of areas with high soil selenium concentrations and will use these areas to produce beef, wheat and broccoli that is enhanced in selenium. Food produced in these regions will be fed to laboratory animals to determine the mechanism of cancer inhibition. These foods will also be fed to humans living in selenium-deficient and selenium adequate areas to determine the specific health benefits of supplementation.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0403407 Year: 01 Project Number: 5450-51000-029-05 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

6. What do you expect to accomplish, year by year, over the next 3 years?

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

9. Scientific Publications:

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404307 Year: 01 Project Number: 5450-51000-029-06 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: AGRICULTURAL PRODUCTION ASPECTS OF HIGH SELENIUM MEAT AND WHEAT

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

2. How serious is the problem? Why does it matter?

3. How does it relate to the National Program(s) and National Program Component(s)?

4. What were the most significant accomplishments this past year?

This report serves to document research conducted under a Specific Cooperative Agreement between ARS and the Department of Animal and Range Sciences, North Dakota State University.

In order to fully characterize factors that influence the accumulation of selenium in wheat and beef, four geographical areas in the Northern Plains have been identified with soils containing low or high or variable concentrations of selenium. Wheat has been planted on each of the sites and the wheat is being sampled at regular intervals throughout the growing season. Forage is being harvested from these sites and will be used in feeding experiments to determine the accumulation of selenium in beef. Soil and soil water samples are being taken at regular intervals to measure geological and geochemical factors associated with the accumulation of selenium in wheat and beef.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

6. What do you expect to accomplish, year by year, over the next 3 years?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404307 Year: 01 Project Number: 5450-51000-029-06 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

9. Scientific Publications:

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404348 Year: 01 Project Number: 5450-51000-029-07 G
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: CHEMICAL FORMS OF SELENIUM IN FOODS

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

2. How serious is the problem? Why does it matter?

3. How does it relate to the National Program(s) and National Program Component(s)?

4. What were the most significant accomplishments this past year?

This report serves to document research conducted under a grant type agreement between ARS and the Department of Chemistry, University of North Dakota.

A major health benefit of selenium is a possible reduction in the incidence of certain cancers. The cancer-suppressive actions of selenium are determined in part by the chemical form that selenium is consumed, therefore determination of the chemical forms of selenium in high-selenium foods is a high priority. Selenium is extracted from broccoli by a variety of methods including hot water, incubation with a protease solution and organic solvents. The solution is chromatographed to separate the different chemical forms which are identified by mass spectrometry. The detected chemical forms of selenium are greatly affected by buffer conditions used during extraction. The primary chemical forms of selenium in broccoli include inorganic forms, methylated forms and amino acid forms. Methods are being developed to fully characterize the forms of selenium in high-selenium wheat and grass.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404348 Year: 01 Project Number: 5450-51000-029-07 G
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

6. What do you expect to accomplish, year by year, over the next 3 years?

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

9. Scientific Publications:

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404351 Year: 01 Project Number: 5450-51000-029-08 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: HEALTH BENEFITS OF HIGH-SELENIUM FOODS TO HUMANS

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

2. How serious is the problem? Why does it matter?

3. How does it relate to the National Program(s) and National Program Component(s)?

4. What were the most significant accomplishments this past year?

This report serves to document research conducted under a Specific Cooperative Agreement between ARS and the Department of Agricultural Chemistry, Oregon State University.

The beneficial actions of selenium depend in part on how selenium is distributed in the body. Selenium is a component of many proteins; some of which have a high priority for selenium when it is limited, whereas others have a much lower priority. Thus, determining the distribution of selenium among selenium proteins may give an indication of the potential health benefits of certain sources of selenium. Plasma from humans, cattle and rats was subjected to two methods of chromatography and individual fractions were collected and analyzed for selenium. Selenium was distributed between selenoprotein P, glutathione peroxidase and albumin. More selenium was in the albumin fraction when the selenium source contained selenomethionine and more was in the selenoprotein P fraction when the selenium source contained methylated selenium (e.g. high-selenium broccoli). These results help explain the increased anti-carcinogenic activity of selenium from broccoli.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404351 Year: 01 Project Number: 5450-51000-029-08 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

6. What do you expect to accomplish, year by year, over the next 3 years?

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

9. Scientific Publications:

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404481 Year: 01 Project Number: 5450-51000-030-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Title: HUMAN MINERAL ELEMENT REQUIREMENTS AND THEIR MODIFICATION BY STRESSORS

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The extent to which dietary mineral excesses, deficiencies and imbalances contribute to the susceptibility and severity of a number of chronic diseases of major health and economic consequence has not been established; the chronic diseases include coronary heart disease, hypertension, stroke, atherosclerosis, and osteoporosis. Additionally, the extent to which mineral element nutrition contributes to these diseases is unknown when nutritional, metabolic, hormonal or physiological stressors are present which could enhance the need, or interfere with the utilization of the mineral elements. There is a need to ascertain the validity of claims that magnesium is of practical concern for maintaining bone and cardiovascular health; that boron, copper, zinc and manganese status affects calcium utilization and metabolism and thus the susceptibility of osteoporosis; and that low and/or high dietary zinc adversely affect copper metabolism resulting in an increased risk to cardiovascular disease.

Studies with human volunteers are being and will be conducted. These studies include examining the effects of varying intakes of zinc at different intakes of dietary copper on lipid profiles, bone status indicators and reactive oxygen metabolism; and whether low magnesium intakes with and without stressors results in a neurogenic inflammatory response leading to oxidative damage that can lead to pathophysiology such as cardiomyopathy, migraine headaches, and abnormal central nervous system function.

2. How serious is the problem? Why does it matter?

Dietary factors, including trace element nutriture, are associated with 5 of the 10 leading causes of death. Among the diseases that are closely linked to diet, the cost for treatment and care in the United States exceeds $200 billion per year. Among the diseases associated with subnormal mineral

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National Program(s): 107 100%

element nutrition, the annual economic cost is estimated to be greater than $80 billion for cardiovascular disease, and $10 billion for osteoporosis. Several mineral elements associated with these chronic diseases including copper, magnesium and zinc have been shown to be routinely low in the diets in the United States. Thus, providing information about requirements and factors that affect those requirements of critical mineral elements should result in policies and programs that improve intakes of these nutrients and thus result in a healthier population, a decrease in the burden of chronic disease, an enhancement in the quality of life, and a diminishment in health care expenditures.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research relates to the National Program 107, Human Nutrition, and specifically to the program component of Nutrient Requirements. The research helps determine mineral requirements that prevent disease and promote health and optimal function throughout life. The major focus is on determining the biochemical and health consequences of suboptimal mineral intakes, with the objective of showing that mineral nutrition influences the major chronic, degenerative conditions associated with aging.

4. What were the most significant accomplishments this past year?

A. None

B. None

C. None

D. This report represents a bridging CRIS; the predecessor CWU was 5450-51000-022-00D HUMAN MINERAL ELEMENT REQUIREMENTS AND THEIR MODIFICATION BY STRESSORS. Since the start date of the bridging CRIS, the metabolic unit portion of a human experiment with the objective of confirming and extending findings showing that a system neurogenic inflammatory response is of primary importance in magnesium deprivation was completed. Samples collected are now being analyzed. Also, additional human volunteers started the metabolic unit phase of an experiment designed to show that a low dietary zinc intake adversely affects copper metabolism resulting in an increased risk to cardiovascular disease.

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National Program(s): 107 100%

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

New bridging CWU; all accomplishments listed above.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY 2002 - A human study will be completed which will confirm that low dietary zinc is an important factor in inducing changes in copper status indicators, and that a combined low intake of copper and zinc results in changes that could lead to increased susceptibility to osteoporosis and heart disease.

FY 2003 - A human study will be initiated to test the hypothesis that magnesium deprivation and a high dietary intake of manganese are of practical nutritional concern because when they are combined the result is biochemical and physiological changes that could lead to pathological consequences to heart, bone and brain function.

FY 2004 - Human studies will be initiated to determine whether nickel status affects the blood pressure response and kidney function of adults consuming high dietary sodium; and whether silicon status of post menopausal women affects bone health and thus is an important factor in the occurrence of osteoporosis.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

None

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

None

9. Scientific Publications:

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0403042 Year: 01 Project Number: 5450-51530-005-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.3.1.1 50%
National Program(s): 107 100%

Title: BIOMARKERS FOR ASSESMENT OF HUMAN MINERAL NUTRITIONAL STATUS AND REQUIREMENTS

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:
1. What major problem or issue is being resolved and how are you resolving it?

Osteoporosis is a common bone disease whose incidence has not been effectively reduced. One out of every two women and one out of eight men over age 50 will have an osteoporosis-related fracture in their life time. Nutritional factors are of particular importance in the prevention of osteoporosis because they can be modified (unlike other risk factors such as genetic predisposition, age, gender). It is generally agreed that calcium and vitamin D are important nutrients for bone health, and supplements containing these nutrients are prescribed widely. However, much less is known about the contribution of suboptimal intakes of other minerals known to be essential for bone development and maintenance (for example, zinc, copper, magnesium) to the incidence of osteoporosis. Also, the effects of large doses of calcium, as found in supplements, on the absorption and utilization of other minerals important to bone health have not been sufficiently determined. Moreover, the effects of common dietary practices like consuming diets high in soy protein versus animal protein on bone metabolism need further elucidation.

As we age, the circulating concentration of an important hormone called insulin-like growth factor-1 (IGF-1) also decreases leading to reduced bone formation and gradual bone loss (this is termed somatopause). It is known that food intake is one of the primary regulators of this bone-building hormone, but the specific effects of varying intakes of trace minerals like zinc, copper and magnesium on this hormone have not been ascertained. Investigations on the effects of different trace minerals on IGF are a new promising area of the potential role of nutrition in prevention of osteoporosis. A series of animal experiments will be conducted to determine how varying intakes of key minerals (e.g., calcium, zinc, copper, magnesium) affect bone health and the IGF proteins. Ovarioectomized rats will be used to simulate the postmenopausal state. In addition to the classical measurements of bone mineral content, density, and mechanical

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National Program(s): 107 100%

properties (e.g., breaking strength), we will monitor changes in bone proteins. The human studies will include calcium supplementation trials with or without other important minerals (zinc, copper, magnesium), and controlled feeding studies to determine the effects of common dietary practices such as eating foods high in soy protein on bone health and to determine if calcium supplementation affects the status of other important minerals in the body. As a part of these studies we will determine how people with different genetic makeup (example, difference in vitamin D absorption or serum IGF-1) respond to calcium supplementation.

2. How serious is the problem? Why does it matter?

The annual cost of healthcare related to osteoporosis is estimated at $14 billion. As the US population ages, the economic cost of osteoporosis is projected to reach $50 billion by the year 2040. It has been estimated that the lifetime risk of fracture exceeds 40% for women and 13% for men. In the elderly, hip fractures are associated with mortality in up to 20% of the cases, with costly long-term nursing home care required for most survivors. Dietary modification is a sensible, practical and economically feasible approach to the prevention of osteoporosis. As trace minerals are known to be essential for bone health, it is important that we determine their specific role in prevention of this devastating disease.

3. How does it relate to the National Program(s) and National Program Component(s)?

This research relates to the National Program 107, Human Nutrition. Specifically, it addresses nutrient requirements, mechanisms of action of nutrients, identification of biomarkers (for the study of bone status), nutrient-nutrient and nutrient-gene interactions, identification of health promoting properties of plant and animal foods, and health promoting intervention strategies for targeted populations.

4. What were the most significant accomplishments this past year?

A. It is known that nutritional status regulates the concentration of serum insulin-like growth factor-1 (IGF-1), a small protein important for bone health; however, the effects of intake of specific trace minerals on serum IGF-1 are not known. A study was conducted (in collaboration with Dr. Lukaski) to test the effects of graded intakes of copper and

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National Program(s): 107 100%

zinc (low, marginal and high) on serum IGF-1 and its relationship to bone strength in growing rats. Our data indicate that low copper intake significantly reduces serum IGF-1, bone density, and bone strength and that serum IGF-1 was significantly correlated with bone density and strength. In previous studies, the adverse effects of low copper intake on bone strength has been attributed to a reduced activity of lysyl oxidase, a copper-dependent enzyme essential for collagen cross linking---our findings indicate that low copper intake leads to weak bones, at least in part, by reducing serum IGF-1 concentration.

B. Recently, the Food and Drug Administration approved a health claim stating that consuming 25 g of soy protein is beneficial to heart health; however, the effects of this dietary practice on bone health are not known. A controlled feeding study of postmenopausal women designed to determine calcium retention (by using calcium-47 as a tracer and whole body scintillation counting) from diets containing meat protein to those in which 25 grams of soy protein have been substituted for meat protein has been approved to begin in October of 2001. The findings from this study will have important implications as they will provide evidence of the effects of this dietary practice on calcium retention and bone health.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

This project was initiated in FY 2000. Over the life of the project, it is expected that our understanding of the role of trace minerals in the determination of peak bone mass and rate of bone loss (including the role of IGF-1 in these processes) will be enhanced. Furthermore, by using clinical trials, it is expected that the data base on the effects of common dietary practices such as consuming soy protein or mineral supplementation on bone metabolism will be expanded.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY 2002: A controlled feeding study will be conducted to compare the effects of consuming soy protein versus animal protein on calcium absorption and bone status of postmenopausal women. A double-blind placebo-controlled supplementation study will be initiated to compare the effects of calcium supplementation with and without other important minerals (specifically zinc, copper and magnesium) on bone

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loss in postmenopausal women. Animal studies will be conducted to examine the effects of consuming marginal amounts of calcium and varying amounts of copper, zinc and iron on bone status and IGF proteins in rats.
FY2003 and 2004: The long-term supplementation trial will continue. Controlled feeding studies designed to compare the calcium retention from vegetable protein vs. meat protein will be conducted. Animal studies investigating the effects and mechanisms of action of trace minerals on the IGF-1 proteins and bone status will be conducted.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Technology has been transferred through a variety of activities by writing articles for the local newspaper ("Soy...the newest way to "bone-up" on good nutrition?"), by speaking to various consumer and stakeholder groups (Farm Credit Services, North Dakota Beef Commission, Retired Federal Employees, Young in Spirit Group) and providing consultation to various media groups (Los Angeles Times and Minnesota Public Television).

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Roughead, ZK (2001)The Body Knows Best? Trends in Endocrinology and Metabolism (In press)
9. Publications:
Roughead, D.A., Lykken, G.I., Hunt, J.R. Controlled high meat diets do not affect calcium retention or indices of bone status in healthy postmenopausal women. Federation of American Societies for Experimental Biology Journal. 2001. v. 15. p. A727.
Hill, K., Lukaski, H., Roughead, F. Low copper intake reduces serum IGF-1 and bone strength. Proceedings of the North Dakota Experimental Program to Stimulate Competitive Research (EPSCoR). 2001. p. 15.

9. Scientific Publications:

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404773 Year: 01 Project Number: 5450-51530-007-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 25% 5.3.1.2 75%
National Program(s): 107 100%

Title: DEVELOPMENT AND EVALUATION OF METHODS FOR THE CLINICAL EVALUATION OF MINERAL NUTRITIONAL STATUS

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Although the essentiality of magnesium, copper and zinc is well established, the general consensus in the mineral research community is that there are currently no adequate clinical tests for evaluating the nutritional status of these elements in humans. Moreover, the lack of accurate diagnostic tests to assess magnesium, copper and zinc status has impeded the development of robust well-founded dietary guidance for these essential elements, and impeded the identification of chronic diseases such as ischemic heart disease and osteoporosis, whose incidence and severity may involve imbalanced or deficient intakes of these essential mineral elements.

The approach to resolving the problem is to feed human volunteers deficient or imbalanced intakes of copper, zinc and/or magnesium, measure variables that can be ethically assessed and are responsive to deficient or imbalanced intakes based on findings from animal models, and compare the values obtained to those obtained when intakes of these elements are balanced and adequate.

2. How serious is the problem? Why does it matter?

Food consumption surveys indicate that a significant number of people in the United States have intakes of copper, zinc and/or magnesium that are below those recommended by the Food and Nutrition Board of the National Academy of Sciences. Yet, pathology caused by deficiencies of these elements is not readily recognized or diagnosed because of the lack of definitive methods for identifying deficient states. Establishment of specific, accurate and cost-effective tests for the measurement of nutritional status will aid in detecting nutritional deficiencies and imbalances in their early stages. Early detection of inadequate trace element status will result in considerable savings in the cost for

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National Program(s): 107 100%

treatment and care of diet-related diseases in the United States that has been estimated to exceed $200 billion per year. Additionally, methods to assess status would be applicable to studies determining human requirements and metabolism of trace elements. Knowledge gained from this research project will facilitate the evaluation of federal food and nutrition programs, and the administration of programs that contribute to the health and well-being of people.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research relates to the National Program 107, Human Nutrition, and specifically to the program component of Nutrient Requirements which states research is needed to identify biomarkers of nutrient intakes and status, nutritional adequacy and disease prediction. The research emphasizes priority objectives of Biomarkers, and Function and Performance, but also can apply to objectives of Mechanism of Action and Nutrient Interactions.

4. What were the most significant accomplishments this past year?

None.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

None.

6. What do you expect to accomplish, year by year, over the next 3 years?

This project will be terminated March, 2002.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

None.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

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National Program(s): 107 100%

None.

9. Scientific Publications:

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

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MINERAL NUTRIENT FUNCTIONS

MANAGEMENT UNIT

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401450 Year: 01 Project Number: 5450-51000-023-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 80% 5.1.3.4 20%
National Program(s): 107 100%

Title: BIOCHEMICAL CONSEQUENCES OF SUBOPTIMAL DIETARY INTAKE OF TRACE ELEMENTS

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Neither the explicit health effects of low dietary intakes of copper, zinc and selenium nor the dietary requirements for these elements for health and optimal performance are well-defined. Because zinc and copper serve as cofactors for a large number of enzymes that catalyze important biochemical reactions, the effects of low zinc or copper intakes on the in vitro activities of these enzymes are paradigms that have been used for estimating human and animal requirements for these elements. Similarly, selenium is important to the activities of several enzymes that protect cells against damaging oxygen radicals. Oxidative damage to cell components resulting from low selenoenzyme activities has been implicated in the pathology associated with selenium deficiency. However, the paradigm of reduced enzymatic activities has not provided sufficient information to account for the biological and health consequences of low dietary intakes of copper, zinc and selenium. Major reasons for this insufficiency are: (1) the lack of knowledge regarding how reductions in the activities of zinc-, copper-, and selenium-dependent enzymes translate into altered cellular and organ function and (2) the lack of knowledge regarding non-enzymatic roles of these elements.

Animal models of copper and zinc deficiencies and cells cultured in media containing various concentrations of zinc, copper, and selenium are used to investigate the various biochemical mechanisms underlying the functional consequences of low zinc and copper intakes and low cellular content of zinc and copper. The influences of copper and zinc deprivation on the synthesis of bioactive molecules, transport mechanisms, transmembrane signaling, mitochondrial function, and mechanisms regulating programmed cell death will be determined. Knowledge based on the descriptions of biochemical mechanisms for the functional outcomes of zinc and copper deficiencies can more precisely define the dietary requirements for zinc and copper for health and optimal performance during all stages of life in men and women.

There are numerous brands of supplements sold in the marketplace that contain large amounts of zinc. At present, these zinc supplements and their contents are not regulated. Certain dietary nutrients such as copper and zinc interact in the gut to inhibit the absorption of each other. However, zinc affects copper absorption and utilization more than copper affects zinc. If a person consumes two to three times the requirement (RDA) of

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National Program(s): 107 100%

zinc, either from food or, more likely, from food supplements for an extended period, copper absorption could be reduced to such an extent that the person may develop a moderate grade copper deficiency. Therefore, there is a need to understand the physiological effects of high zinc intakes on copper absorption and utilization. A human intestinal cell model is used to determine the basic mechanisms involved in the copper-zinc absorption interaction. This information can be used to help set standards for zinc contents in food and food supplements, and for use in making recommendations for copper intake when dietary zinc might be in excess.

Selenium can also interact with other dietary or environmental trace elements. These interactions may interfere with the anticancer function of selenium by impairing its role in triggering the death of cancer cells. A human leukemia cell model will be used to investigate the basic mechanisms through which interactions between selenium and other dietary or environmental trace elements influence programmed cell death. This information will be useful for designing dietary strategies to establish multiple mineral requirements to decrease the risk of cancer.

2. How serious is the problem? Why does it matter?

Criterion for establishing the adequate intakes for copper are based on plasma copper concentrations, serum ceruloplasmin concentration, erythrocyte superoxide dismutase activity and plasma copper concentration. Adequacy for zinc intake is based on balance and physical growth to zinc supplementation. However, these criterion are not sufficient to establish recommended dietary intakes of copper or zinc for disease prevention. The establishment of criteria for intakes of copper and zinc that prevent or delay the development of degenerative diseases of the cardiovascular, muscular and nervous systems requires knowledge regarding the effects of low copper or zinc intakes on specific cellular mechanisms that when perturbed lead to pathological states. Knowledge regarding the cellular mechanisms leading to negative health effects of low zinc and copper intakes can provide a basis for recommending dietary requirements that can slow or ameliorate the development of degenerative diseases. Dietary interventions based on this knowledge could reduce the $200 billion spent yearly in the United States for treatment of diseases that are strongly associated with diet.

Intake of a small excess of zinc in the form of a supplement could easily promote or induce a mild to moderate copper deficiency. Copper is an essential nutrient for myriad physiological and biochemical processes, many of which are involved with development and function of the nervous system in the fetus. Although zinc is also required for development, it is extremely important to know the precise balance between the two nutrients so that an excess of one can be counterbalanced by the proper intake of the other.

Epidemiological and human studies have revealed that selenium is an effective anticancer agent. However, interactions between arsenic and

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National Program(s): 107 100%

selenium could reduce the anticancer properties of selenium. It has been suggested that the cancer risk from arsenic in the drinking water of the United States is comparable to that of environmental smoke and radon in homes. Thus, it is important to understand how the interaction between selenium and arsenic or other elements affects the mechanism involved in initiating and regulating cell death. Such information will help establish dietary selenium requirements for optimizing the anticancer effects of selenium in relation to dietary intakes of arsenic and other elements that interact with selenium.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research relates to National Program 107, Human Nutrition (100%) and the nutrient requirements component of the program. Priority objectives addressed by the research are biomarkers, mechanism of action, nutrient interactions, environmental factors, and function and performance. This research will develop information about the effects of zinc, copper and selenium deficiencies on biochemical functions that will facilitate the detection of marginal deficiencies of these elements and define their dietary requirements for health, development and optimal performance throughout the life cycle. The research will also provide information that can be used to assess the risk of chronic disease from subclinical zinc, copper and selenium deficiencies.

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishment during FY 2001:
Excess zinc in the diet can interact with other essential minerals such as copper and iron to prevent their absorption and lead to deficiencies. Studies were done to determine if specific functions of copper transport were affected by zinc to reduce copper uptake and transport in intestinal cells. By using the Caco-2 cell in culture as a model, it was shown by Western blotting techniques that at least one of the copper transport proteins called Wilson's disease protein was not affected by zinc; however, in the rat model, the mRNA for the Menkes transporter was reduced by one-half in the gut cells when the animals were fed high zinc diets. Impact: This work shows that zinc might affect the abundance of the copper transporters, which would suggest a mechanism for the initiation of low copper status in humans and animals that consume excess zinc in their diets.

B. Other Significant Accomplishments(s):
Heme oxygenase is an important antioxidant enzyme and regulator of heme iron metabolism, but it is not known whether oxidative stress caused by copper deficiency increases heme oxygenase activity by increasing heme oxygenase-1 expression or increasing NADPH:cytochrome P450 reductase

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expression. A study was performed at the Grand Forks Human Nutrition Research Center to examine the effects of copper deficiency on the hepatic expression of heme oxygenase-1 and NADPH:cytochrome P450 reductase and to determine if mitochondrial production of reactive oxygen species could be involved in the expression of these enzymes. It was found that copper deficiency had no effect on hepatic NADPH:cyrochrome P450 reductase content but did increase heme oxygenase-1 content in a manner that was inversely related to the activity of mitochondrial respiratory complex I activity. Impact: This work identifies impaired mitochondrial respiratory activity as an outcome of copper deficiency that increases oxidative stress, activates the induction of stress-related proteins and increases the risk of degenerative diseases associated with increased release of heme iron and mitochondrial dysfunction.

Selenium is a dietary mineral that has the potential to prevent cancer and other diseases related to cell cycle perturbations, but the dietary levels of selenium needed for disease prevention are largely unknown. At the Grand Forks Human Nutrition Research Center, we used a human leukemia HL-60 cell to study the effects of selenium on cell growth. Our data from the study suggest a novel role for selenite/seleno-methionine at physiological concentrations in the up regulation of numerous cell cycle related genes (c-Myc, cdk2, cyclin C, PCNA, cdk1, cdk4, cyclin B and cyclin D2) and total cellular kinase proteins, which are critical in cell cycle progression particularly in G2/M transition. Impact: Elucidation of mechanisms through which selenium controls and regulates the cell cycle can lead to a better understanding of the nature of selenium-essentiality and help establish the selenium requirement for disease-prevention.

C. Significant Accomplishments/Activities that Support Special Target Populations:
None.

D. Progress Report.
None.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

The effect of marginal copper deficiency during pregnancy on the expression of the alpha, beta, and gamma isoforms of protein kinase C in neonatal rat brain was examined. Copper deficiency reduced the rate of expression of the protein kinase C isoforms during the three weeks following birth and led to significant reductions of protein kinase C beta in the hypothalamus and protein kinase C gamma in the hypothalamus and cerebellum. Impact: Protein kinase C expression is a determinant of brain development. Impairment in the expression of protein kinase C isoforms may eventually explain how neurological function and intellectual development

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are affected in the offspring of mothers who are subclinically copper deficient during pregnancy and the perinatal period.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY 2002-03: Examination of the effects of copper deficiency on the production of reactive oxygen species by liver, heart, brain and muscle mitochondria will be continued. It will be determined if the altered redox state of cells caused by increased mitochondrial production of reactive oxygen species alters oxidant sensitive signaling pathways leading to apoptosis.

FY 2002-03: Studies to determine if maternal copper deficiency impacts mitochondrial function in liver, heart, muscle, and brain of neonates. The offspring of copper-deficient dams will be followed as they age to determine if mitochondrial dysfunction caused by maternal copper deficiency is irreversible.

FY 2002-03: Studies will be designed to further characterize the effect of zinc on the physiological function of the cell membrane transporters for copper. Zinc seems to affect the relative abundance of the MNK protein and its mRNA; however, it is not known exactly how zinc might have this effect. The question to be answered, is zinc involved in transcription factors that govern mRNA production, and/or is the efficiency mRNA translation affected by zinc? The answer to this question would significantly advance our knowledge about the regulation of transport of copper and about how cells regulate the accretion of trace elements in general.

FY 2002-03: Studies will continue to characterize the effect of zinc on the regulation of zinc transporter proteins. High zinc down regulates the rate of zinc uptake into and transport across intestinal cells. Is this because the transporter protein is not being manufactured or is the transporter itself being deactivated by its own substrate? Answers to these questions would significantly advance our knowledge about the regulation of transport of zinc and about how cells regulate the accretion and distribution of trace elements in general.

FY 2002-03: Studies will continue to determine the mechanisms of how the concentration of dietary zinc regulates food intake. State-of-the-art feeding monitors will be used to study feeding characteristics in zinc deprived rats and the patterns correlated with bioactive peptides that seem to be regulated by zinc dependent enzymes. These include neuropeptide Y and cholecystokinin. Food intake is very responsive to dietary zinc intake, and is a good model to study food intake regulation in general.

FY 2001-02: Define the molecular basis of selenium and copper in cell cycle and apoptosis in cultured cells. Information from the investigation will advance our knowledge about the importance of selenium at molecular level when cells are under a stress condition.

FY 2001-02: Gene transcriptional regulation of copper/zinc superoxide dismutase and manganese superoxide dismutase in response to nutritional mineral trace elements will be examined with a gene reporter system. The

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Accession: 0401450 Year: 01 Project Number: 5450-51000-023-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 80% 5.1.3.4 20%
National Program(s): 107 100%

results of the study will shed light upon the roles of mineral trace elements in gene transcriptional regulation of anti-oxidative enzymes.

FY 2001-02: The effects of marginal deficiencies of calcium, zinc, and iron on cadmium availability from wheat products will be examined. Pasta will be the first wheat product studied in the series. The minimal concentrations of food calcium and iron that provide a protective effect against excessive cadmium absorption will be determined.

FY 2002-03: Investigations will be conducted to determine whether calcium, zinc and iron content in food crops can be altered to either take up less cadmium and/or to increase their concentration of calcium and iron in order to provide a natural deterrent to cadmium absorption by the consumer but, at the same time, maintain their natural nutritional value.

FY 2002-03: Determine the impact of mineral status on the biological activity of zinc finger transcriptional factors, which could lead to development of the molecular bio-marker for optimal mineral intake and disease prevention.

FY 2003-04: Investigations will be conducted to determine if oxygen radicals generated within mitochondria during copper deficiency can cause permanent mitochondrial dysfunction by inducing mutations in mitochondrial DNA. It will be determined if different types of dietary carbohydrates, lipids, and antioxidants can potentiate or ameliorate oxidative damage caused by copper deficiency.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Results from studies are published in peer-reviewed scientific journals. Upon publication, the methodology, data, and interpretations of the data are immediately available to scientists and other interested parties. In addition, experimental results and educational information pertinent to public interests are made available through the local newspaper, The Grand Forks Herald, and nationally and internationally through the Center's Web Site on the Internet. During FY2001, the following information was transferred to the public through articles published in the Grand Forks Herald: "Nutrition and the human genome: What possibilities" by W.T. Johnson and "Colorful plate of foods offers more appeal than you think" by H. Zeng.

A seminar titled "The biological role of copper in signal transduction: From platelets to cancer" was presented by W.T. Johnson at the University of North Dakota School of Medicine.

A poster titled "Copper deficiency in HL-60 cells increases their susceptibility to apoptosis when treated with antioxidants" by L.J. Sumner and W.T. Johnson was presented at Frank Low Research Day at the University of North Dakota School of Medicine.

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National Program(s): 107 100%

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

None.

9. Scientific Publications:

01. Reeves, P.G., Chaney, R.L. Mineral status of female rats affects the absorption and organ distribution of dietary cadmium derived from edible sunflower kernels (Helianthus annuus L.) Environmental Research Section A. 2001. v. 85. p. 215-225.

02. Momcilovic, B., Reeves, P.G. Idiorrhythmic zinc dose-rate induction of intestinal metallothionein in rats depends upon their nutritional zinc status. Journal of Nutritional Biochemistry. 2001. v. 12. p. 225-234.

03. Huang, Z.L., Failla, M.L., Reeves, P.G. Differentiation of human U937 promonocytic cells is impaired by moderate copper deficiency. Experimental Biology and Medicine. 2001. v. 226(3). p. 222-228.

04. Reeves, P.G. Mineral nutrient status and the bioavailability of cadmium from natural food sources. Syers, J.K., Gochfeld, M., editors. SCOPE, Paris, France. Proceedings of the SCOPE Workshop on Environmental Cadmium in the Food Chain: Sources, Pathways, and Risks. Sept 13-16, 2000. p. 82-86.

05. Sumner, L.J. Johnson, W.T. Copper deficiency in HL-60 cells increases their susceptibility to apoptosis when treated with antioxidants. Abstract presented at Frank Low Research Day, April 19, 2001, University of North Dakota.

06. Johnson, W.T. Mitochondrial oxidative stress may contribute to the induction of hepatic heme oxygenase-1 in copper deficient rats. The FASEB Journal. 2001. v. 15(5). Abstract p. A271. Presented by W.T. Johnson at the Experimental Biology 2001 meeting, Orlando, FL, March-April 2001.

07. Sumner, L.J., Johnson, W.T. Copper deficiency in HL-60 cells increases their susceptibility to apoptosis when treated with antioxidants. The FASEB Journal. 2001. v. 15(5). Abstract p. A271. Presented by L.J. Sumner at the Experimental Biology 2001 meeting, Orlando, FL. March-April 2001.

08. Zeng, H. The effect of arsenic on the selenium-induced cell death pathway. The FASEB Journal. 2001. v. 15(5). Abstract p. A604. Presented by H. Zeng at the Experimental Biology 2001 meeting, Orlando, FL. March-April 2001.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0403340 Year: 01 Project Number: 5450-51000-023-01 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Title: CORRELATION BETWEEN SPERM MOTILITY AND HEAVY METAL STATUS OF ANIMALS AND HUMANS

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

In the equine breeding industry, cryopreservation of the semen has not been perfected to the point where the freeze-thaw cycle does not cause low viability and sperm count, and/or low sperm motility. This results in reduced ability of the sperm to fertilize the egg. Human male fertility studies have shown that the trace element content of the semen, especially zinc, is an important factor that helps control sperm count, viability, and motility. We question whether this could be part of the cause, if not the sole cause, of similar problems in horses. In addition, could other trace elements at either essential and/or toxic concentrations, be part of the causative factor?

The initial phase of this study will be to collect body fluids such as semen, plasma, and urine from a population of stallions and measure the trace element content to ascertain the physiological status of each element. Human samples also will be collected. We will determine if there is a correlation between low to high concentrations of the elements and sperm viability, motility, and survival time during cryopreservation. The trace elements to be analyzed include calcium, zinc, copper, iron, selenium, cadmium, chromium, and others that might be of interest.

2. How serious is the problem? Why does it matter?

As new reproductive technologies have become available to the horse breeding industry, it has become apparent that there are unexpected problems affecting semen preservation and sperm viability. At present there is no good way to ship frozen semen to maintain high sperm viability, thus causing a major loss of the product at great cost to the breeders. Some of these problems could be related to the mineral content of the semen. There are viability problems with human sperm as well that could have a similar etiology.

3. How does it relate to the National Program(s) and National Program Component(s)?

This research will develop information about the effects of various

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National Program(s): 107 100%

mineral elements on the viability, motility, and biochemical functions of animal and human sperm. In addition, this research will allow us to determine safe and adequate intakes of mineral elements for optimal health and bodily functions. Thus, the research relates to National Program 107, Human Nutrition (100%) and the nutrient requirements component of the program. Priority objectives addressed by the research are biomarkers, mechanism of action, nutrient interactions, environmental factors, and function and performance.

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishment during FY 2001:
The first phase of this project got under way late in FY 2001. The past year was spent analyzing over 3000 samples for various minerals. The results of these analyses have yet to be analyzed.

B. Other Significant Accomplishments:
None.

C. Significant Accomplishments/Activities that Support Special Target Populations.
None.

D. Progress Report.
None.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

None. This project is still in the early stages.

6. What do you expect to accomplish, year by year, over the next 3 years?

This project was initially set up only for a two year duration (FY 2001-2002). During this period, we will analyze numerous urine, semen, and blood samples from humans and horses to determine relationships between mineral content and in vitro function of mineral elements.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

None.

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Mode Code: 5450-20-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

None.

9. Scientific Publications:

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404448 Year: 01 Project Number: 5450-51000-025-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.1.3.4 25%
National Program(s): 107 100%

Title: DIETARY TRACE ELEMENTS AND PHYSIOLOGY OF THE CARDIOVASCULAR AND RELATED SYSTEMS

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Dietary copper deficiency causes biochemical deficits and structural damage to the cardiovascular system (heart and circulation). Although progress is being made in identifying defects in cardiovascular physiology (function) in copper deficiency, the mechanisms for these functional changes are not completely clear. The known functional changes are not related in a clear fashion to the known structural and biochemical changes. Additionally, because most of the studies on dietary copper have not been done in humans or with intakes consistent with human consumption, the relationship of such findings to human health is not clear. The approaches to resolving this problem include:

A. Determination of functional changes in blood vessels and, in particular, clarification of changes in signal transduction pathways in smooth muscle and endothelium caused by dietary copper deficiency. Relevant studies will be performed with isolated vessels, on isolated organs and on whole animals. The ultimate goal is to determine the contribution of adequate copper nutrition to maintenance of blood flow to organs and to maintenance of blood pressure.

B. Identification of functional changes in the heart and their relationship to metabolic and biochemical alterations caused by trace element (copper) deficiencies. The focus will be to determine coronary blood vessel and cardiac muscle vulnerability to physiologic and metabolic stressors including, but not limited to, adrenaline stimulation and simulated heart attack (cessation and re-starting of blood flow to the heart). Isolated heart and whole animal models will be used.

C. Elucidation of general biochemical mechanisms of damage caused by copper deficiency. Oxidative stress continues to be a strong, although somewhat equivocal, candidate as a mechanism for generalized damage. Damage by oxidative mechanisms will be compared with that caused by another mechanism, glycation. The aim is to attempt to relate known enzymatic, metabolic and hormonal changes to the deterioration of function that occurs in copper deficiency. Various organs will be tested, but the primary focus will be on the heart and blood.

2. How serious is the problem? Why does it matter?

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National Program(s): 107 100%

Studies to date have indicated that dietary copper deficiency has considerable potential for contributing to chronic disease (for example, ischemic heart disease, atherosclerosis, high blood pressure) and the debilitating effects of aging. Experimental evidence indicates that a third or more of the American population may be consuming less than the Estimated Safe and Adequate Dietary Intake of copper set by the Food and Nutrition Board of the National Academy of Sciences. Research will provide information that will be used to set recommendations for dietary copper based on a reduction in risk of chronic disease, particularly of the heart and blood vessels.

3. How does it relate to the National Program(s) and National Program Component(s)?

These studies directly contribute to National Program 107 (100%). Relative to the new Human Nutrition Action Plan, these studies will address elements of Performance Goal 3.1.1 Human Nutrition Requirements, with specific emphasis on identifying potentially beneficial mechanisms of action of trace elements and characterizing the role of trace elements in achieving optimal physiologic function.

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishment during FY 2001:
Dietary copper deficiency has been shown to cause impaired contraction in hearts when contractile force is measured in the intact heart. To determine the contribution of individual heart muscle cells to this contractile function, cells were isolated and their ability to shorten was measured under a microscope. Unexpectedly, heart muscle cells from copper-deficient rats contracted more strongly than those of copper-adequate animals. This indicates that the weak contraction of whole hearts is caused by a defect in some other part of the heart, probably the connective tissue, and that the improved contraction of heart muscle cells is caused by compensation for the impaired parts of the heart. This indicates that alteration of heart function by copper deficiency is a complex phenomenon and that further study is clearly required to delineate the importance of copper nutrition to cardiovascular function. (Collaborator - J. Ren, University of North Dakota).

B. Other Significant Accomplishments:
Immune function is known to be disrupted in dietary copper deficiency. We examined the possible consequence of this impairment on the lung when acute lung infection or trauma was simulated by infusion of immune complexes. Indeed, lung damage resulting from this infusion was found to be exaggerated by dietary copper deficiency. However, the increased damage was not associated with alteration of immune cells or their function, but rather by elevation of enzymes called matrix metalloproteinases. The fact

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National Program(s): 107 100%

that these enzymes are also elevated in chronic obstructive lung diseases and in adult respiratory distress syndrome (ARDS) suggests that proper copper nutrition may be important in ameliorating these lung pathologies. (Collaborators - A.B. Lentsch, D.A. Schuschke, University of Louisville).

C. Significant Accomplishments/Activities that Support Special Traget Populations.
None.
D. Progress Report.
None.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

We have shown that the concentrations of two important chemical messengers, nitric oxide and cyclic GMP, are elevated in copper-deficient hearts, which suggests their possible role in reduction of heart contractile force in copper deficiency. A subsequent study showed that the amount of one of the enzymes that produces nitric oxide, inducible nitric oxide synthase, is also elevated in copper deficiency, which suggests an elevated genetic expression of this enzyme. These studies help to define the molecular basis for impaired heart function when dietary copper is restricted.

Strong evidence was found supporting the view that glycation, the undesirable binding of sugar to proteins, is enhanced in dietary copper deficiency. Blood analysis revealed the presence of glycated hemoglobin and fructosamine (blood proteins with sugar bound to them) as well as pentosidine (a product of blood protein damaged by glycation). Because glycation is a process that is increased in diabetes and aging, this finding suggests that reduced copper intake may worsen the consequences of these two conditions.

Measurements of heart and blood vessel function in copper deficient animals helped to show that, although cardiac output was not altered by copper deficiency, blood vessel resistance was reduced and volume of blood ejected per beat (stroke volume) of the heart was elevated; the higher stroke volume may contribute to the pathologically greater size of copper-deficient hearts. These and succeeding physiological measurements will help to characterize heart function in dietary copper deficiency.

Studies with collaborators on the effect of copper deficiency on blood clotting function have shown that the aggregation of blood platelets to one another was increased and that adhesion of platelets to blood vessel endothelial cells was reduced. Further, these findings were associated with an alteration of two platelet clotting factors, fibrinogen and von Willebrand factor. These studies emphasize the importance of dietary copper to prevent bleeding.

Another collaborative study found that the dilation of blood vessels in

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National Program(s): 107 100%

response to an inflammatory agent was exaggerated in copper-deficient rats. By use of appropriate blocking agents, the potential mechanism(s) responsible for this change were delineated. This study shows the importance of proper copper intake in mediating the body's inflammatory response to injury.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY 2002: We will examine whether and to what extent the nitric oxide signal transduction pathway is involved in the altered function of copper-deficient hearts; this will include examination of nitric oxide effects on contractile function and mitochondrial respiration as well as cardiac genetic expression of the enzymes that produce nitric oxide in copper-deficient hearts. FY 2003: Research will be aimed at examining the relationship between the alteration of blood vessel function and regulation of blood pressure in copper-deficient animals. This will focus on the known alteration of nitric oxide-dependent mechanisms as well as mechanisms indirectly affected by altered nitric oxide metabolism. FY 2004: Research will be directed toward determining whether a direct association can be made between formation of advanced glycation end-products and altered cardiovascular function of copper deficiency; examination of heart and blood vessel function will be made in the presence of known inhibitors of glycation. Because a positive finding in the latter study would suggest altered carbohydrate metabolism, we will examine the role of the pancreas in initiating cardiovascular effects of dietary copper deficiency; this would include examination of the role of nitric oxide on pancreatic function (i.e., hormone release)and whether the resulting alteration of carbohydrate metabolism can be shown, by functional testing, to account for cardiovascular effects.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Information was transferred to the public through an article in the nutrition section of The Grand Forks Herald newspaper entitled "What makes nutrients essential?" July, 2001.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Nothing to report.

9. Scientific Publications:

01. Wold, L.E., Saari, J.T., Ren, J. Isolated ventricular myocytes from copper-deficient rat hearts exhibit enhanced contractile function. American Journal of Physiology Heart and Circulation Physiology. 2001. v. 281. p. H476-H481.

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National Program(s): 107 100%

Publications: (Continued)

02. Lentsch, A.B., Kato, A., Saari, J.T., Schuschke, D.A. Augmented metalloproteinase activity and acute lung injury in copper-deficient rats. American Journal of Physiology Cellular and Molecular Physiology. 2001. v. 281. p. L387-L393.

03. Gobejishvili, L., Saari, J.T., Adeagbo, A.S.O., Schuschke, D.A. Dietary copper deficiency increases iNOS-mediated vascular relaxation in rat aorta. The FASEB Journal. 2001. v. 15(5). Abstract p. A272. Presented by L. Gobejishvili at the Experimental Biology 2001 meeting. Orlando, FL. March-April 2001.

04. Schuschke, D.A., Saari, J.T., Kato, A., Lentsch, A.B. Copper deficiency enhances acute lung injury and metalloproteinase activity in rats. The FASEB Journal. 2001. v. 15(5). Abstract p. A1097. Presented by D.A. Schuschke at the Experimental Biology 2001 meeting. Orlando, FL. March-April 2001.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0404210 Year: 01 Project Number: 5450-51000-027-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 90% 5.1.4.3 10%
National Program(s): 107 100%

Title: MINERAL ELEMENT NUTRITION, NEUROPSYCHOLOGICAL FUNCTION AND BEHAVIOR

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The consequences of mild to moderate mineral deficiencies for neuropsychological function and behavior are largely unknown. This represents a serious problem because suboptimal mineral intakes and status have been linked to chronic disorders such as depression and dementia. Further, national nutrition surveys indicate that dietary intakes of several essential minerals are less than recommended in many segments of the U.S. population and existing data are frequently inadequate or unavailable to make recommendations based on functional outcomes. This project addresses the need for increased experimentally derived knowledge leading to a better understanding of the relationships among mineral element nutrition, neuropsychological function and behavior. Such knowledge is critical when making recommendations for mineral intakes that will facilitate optimal neuropsychological health and performance throughout the life span in all segments of our population. Behavior is unique as a criterion for establishing nutritional adequacy because it represents the functional integration of all biological systems, including compensatory mechanisms that often determine the practical importance of a nutritional deficit or excess.

Neuropsychological and behavioral consequences of mild and moderate deficiencies in biologically essential mineral elements are determined with the goal of improving health, work and school performance, and sense of well-being in the population. Specifically, studies are designed to determine: the role of mineral elements in cognition (i.e., attention, perception, learning, memory and reasoning) and spatial and motor skills; the effect of mineral nutrition on mood states and emotional and social adjustment; the impact on nutrition-behavior relationships of potential mediating factors, including environmental and endogenous stressors like noise, temperature, sleep duration and quality, and menstrual and menopausal symptoms; and, the effect of mineral nutrition on electro-physiology indexing brain function to gain insights into the mechanisms for nutritional effects on performance and sense of well-being. New methods and technologies are developed to increase efficacy of behavioral assessments and promote their use by other nutrition scientists. Studies of healthy adults and children are complemented by animal studies.

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National Program(s): 107 100%

2. How serious is the problem? Why does it matter?

Findings obtained during the past 40 years indicate that the mineral elements boron, copper, iodine, iron, magnesium, manganese, selenium and zinc likely are important for normal neuropsychological function and behavior of adults and children. However, previous studies have yet to establish the reliability of effects of graded mineral intakes on behavior or to adequately characterize the relationship between mineral element nutrition and brain function and cognition. Such information is critical to characterizing the mechanisms responsible and is needed to apply findings to real-world problems. To respond to intense public interest in the relationship between nutrition and performance, and potentially to improve public health, productivity and sense of well-being, there is a great need to increase our knowledge of the functional consequences of graded intakes of mineral elements, and especially the consequences of marginal intakes common in many segments of the population. Food consumption surveys indicate that intakes of calcium, copper, iron, magnesium and zinc are significantly below the RDA or ESADDI for large segments of the adult population in the United States and worldwide, and many reviews have concluded that mild-to-marginal deficiencies in these and other mineral elements are particularly likely in the groups targeted by this research (e.g., women, children, elderly). Further, increased knowledge of the relationship between mineral element nutrition, neuropsychological function and behavior is needed for a more complete determination of nutrient requirements, establishing recommended dietary intakes, and evaluating the efficacy and adverse effects of taking dietary supplements, a multi-billion dollar industry in the United States.

3. How does it relate to the National Program(s) and National Program Component(s)?

This research relates to program 107, Human Nutrition (100%). The program component most directly addressed by this project is Nutrient Requirements, with objectives including identifying and developing indicators of function and performance that are sensitive to changes in nutrients and bioactive components of the diet, and characterizing the role of nutrients and other dietary components in achieving and maintaining optimal physiologic and psychologic function and performance. This research also addresses the objectives to determine the functional impact of interactions among dietary constituents and among nutrients and lifestyle, environmental and genetic factors. Products of this research also relate to the program components, Relationship between Diet, Genetics and Lifestyle and the Risk for Chronic Disease, and Health Promoting Intervention Strategies for Targeted Populations.

The determination of dietary requirements for optimal cognitive function and performance has been identified as a national need. This research

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furthers ARS objectives by directly evaluating, under highly controlled conditions, the effects of mineral element nutrition on neuropsychological function and behavior of adults, including the elderly, and in children and adolescents. This research facilitates the detection of mild mineral deficiencies and helps define dietary mineral requirements to develop and maintain health and optimal function throughout the life cycle. Examining the combined effects of nutritional insults and exogenous and endogenous stressors offers insights into ways to improve performance in work and school, and in other situations with a high demand. Understanding the true role of mineral element nutrition in neuropsychological function and behavior also helps individuals and groups to more knowledgeably evaluate nutrition claims, and promotes healthy and cost-efficient dietary behavior.

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishment during FY 2001:
Although women of child-bearing ages frequently have inadequate iron (Fe) and zinc (Zn) status, the importance of Fe and Zn nutrition for cognitive function of adult women has not been systematically investigated. A computerized battery of neuropsychological tasks (attention, perception, memory, reasoning and psychomotor function) was administered to 78 women (aged 19-41 y) prior to and following 8 weeks double-blind cross-over treatment with either 30 mg Zn/d or 30 mg Fe/d. Contrasted with a micronutrient only control group, the group treated with Zn showed improved eye-hand coordination measured by percent time-on-target in a tracking task, and reasoning, measured by reaction time in an oddity task, while treatment with Fe improved eye-hand coordination (tracking) and visual perception, as measured by accuracy on a set comparison task. Performance on other tasks in the battery (tapping (simple motor speed), continuous performance task (attention), visual search (perception), word recognition (verbal memory), and face-name recognition (memory)) was not significantly affected by Fe or Zn treatments, or by treatment order or use of oral contraceptives. Findings provide new information about the functional importance of Fe and Zn for menstruating women and may impact future dietary recommendations for Fe and Zn in this population.

B. Other Significant Accomplishments:
None.

C. Significant Accomplishments/Activities that Support Specific Target Populations:
Placed into service a Mobile Nutrition Research Laboratory to conduct nutrition studies with Native Americans living in the Northern Plains Area, with rural populations, and with other specific targeted groups who could not otherwise participate in nutrition research (children, adolescents and the elderly). This mobile laboratory will support research projects designed to determine in these at-risk populations the relationships

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National Program(s): 107 100%

between mineral nutrition and chronic diseases such as obesity, diabetes and dementia, and between mineral nutrition and physiological and behavioral function.

D. Progress Report:

Planned and initiated a field study of the relationship between zinc intakes and status, body composition, cognitive and adaptive function, and psychoeducational performance in children, aged 12-14 years, attending local middle schools. Zinc intakes are manipulated by food fortification and the mobile laboratory (see 4.C. above) is being used to make all assessments (dietary intake, health status, immune function, cognitive function, psychoeducational performance, body composition and physical fitness). Findings will help determine needed zinc intakes to promote optimal physical and behavioral development of adolescents at-risk for zinc deficiency because of rapid growth.

Implemented a study to determine in mature rats the interactive effects of increased glucocorticoid concentrations and restricted zinc intakes on hippocampal damage and working and reference memory, activity and emotionality. Previous studies in rats indicate that environmental and other stressors increase glucocorticoid concentration in blood, damage cells in the hippocampal region of the brain and may impair memory. Zinc deficiency has also been linked to hippocampal damage, memory impairments and hyperemotionality. Findings will indicate whether zinc deficiency increases risk of memory impairments in response to stress and, conversely, whether adequate zinc intakes protect against such impairments.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

This is a new project replacing previous project, Mineral Element Nutrition, Neuropsychological Function and Behavior (CRIS No. 5450-51000-019-00D), terminated 04-11-01.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY 2002: Complete field study of the relationship between zinc intakes and status, body composition, cognitive and adaptive function, and psychoeducational performance on children, aged 12-14 years, attending local middle schools. Zinc intakes will be manipulated by food fortification or supplementation. A new mobile research laboratory will be used to make all assessments. Findings will help determine needed zinc intakes to promote optimal physical and behavioral development of adolescents at risk for zinc deficiency because of rapid growth.

Determine the individual and combined effects of dietary copper and zinc intakes on cognitive function, particularly memory, and brain electrophysiology of healthy postmenopausal women participating in an

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National Program(s): 107 100%

ongoing controlled metabolic unit study. Findings will help determine needed intakes of copper and zinc for optimal cognitive and brain function in older individuals, and to verify previously observed interactive effects where moderately high zinc intakes impaired some aspects of memory in individuals with low copper intakes.

Complete study to determine in mature rats the interactive effects of altered glucocorticoid concentrations and dietary zinc intakes on hippocampal damage and working and reference memory, activity and emotionality. Previous studies in rats indicate that environmental and other stressors increase glucocorticoid concentration in blood, damage cells in the hippocampal region of the brain and may impair memory. Zinc deficiency has also been linked to hippocampal damage, memory impairments and hyperemotionality. Findings will indicate whether zinc deficiency increases risk of memory impairments in response to stress and, conversely, whether adequate zinc intakes protect against such impairments.

Determine the relative efficacy of different biochemical forms of selenium supplementation on central nervous system function and behavior in mature rats. Findings will facilitate future research with humans and may be used to help determine needed intakes of selenium to achieve optimal performance.

FY 2003: Determine the effects of dietary magnesium intakes on brain electrophysiology, sleep quantity and quality, and activity levels of healthy postmenopausal women participating in a controlled metabolic unit study. Findings will help determine whether magnesium has a role in maintenance of normal sleep patterns and the mitigation of symptoms associated with sleep disorders and deprivation.

Plan and implement an intervention study with Guatemalan children aged 6-9 years to determine whether supplementation or food fortification with vitamin B12 will improve cognitive and psychomotor skills, adaptive function and school performance. This research will be a follow-up to a recently completed study showing a correlation between B12 status and these behavioral measures in this at-risk population.

Plan and initiate a field study to determine the nutritional and health status of local institutionalized and non-institutionalized elderly. Nutritional assessment will focus on mineral nutrition, particularly zinc and magnesium. Brain and cognitive function, mood states, sleep quantity and quality, and physical activity will also be measured and related to nutritional status. Findings will be used to facilitate future intervention studies with this at-risk population.

FY 2004: Plan and initiate an epidemiological study to determine the relationship between mineral element nutrition, incidence of disease, physiologic and psychological function and behavior in Native Americans served by the Aberdeen Area Indian Health Services. A mobile research laboratory will be used to make all assessments. Findings will facilitate future intervention studies with this at-risk population.

Determine the relative benefits for cognitive function of feeding selenium-enhanced meat, wheat and broccoli to adults in the Peoples

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National Program(s): 107 100%

Republic of China. This research is scheduled as part of a recently funded, long-term project on selenium bioavailability and function.

Determine the efficacy of copper and iron supplementation for sleep quantity and quality in healthy young adults living in the local community, and for relief of depression in clinically diagnosed institutionalized and free-living adults. Results will validate previous findings of a relationship between status indicators of these minerals and sleep morphology and depressive symptoms.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

As member of the DRI Panel on Micronutrients (Food and Nutrition Board, Institute of Medicine, National Academy of Sciences), reviewed and interpreted scientific literature and expert testimony on arsenic, boron, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc nutrition for determination of the new Dietary Reference Intakes for the U.S. population. Contributed to the formulation of DRIs and preparation of draft report submitted for NAS review, released in January 2001.

Presented the findings of the Panel on Micronutrients for arsenic, boron, nickel, silicon and vanadium at an invited address at Experimental Biology 2001 in Orlando, FL, April 2001. Presented the framework underlying the new Dietary Reference Intakes and findings of the Panel on Micronutrients to nutrition professionals in an invited address to the Chicago Nutrition Association, Chicago, IL, May 2001.

To meet the need for valid yet inexpensive and easy-to-use procedures to routinely assess the relationship between nutrition and behavior, a computer software package and associated procedures were developed to automate the administration of standardized neuropsychological tasks designed to assess a variety of cognitive processes (e.g., perception, attention, learning, memory and reasoning) and psychomotor and spatial skills. Initially designed for English speaking adults, tasks and instructions have recently been adapted for use with children, adolescents, elderly, Native Americans, non-English speaking persons, and other groups at risk for mineral deficiency (e.g., athletes). This technology and related methods represent significant contributions to research on the neuropsychological and behavioral effects of nutritional deficiencies and supplementation. Users of this technology are researchers in private industry and in state and federal governments, domestic and foreign. Currently in progress are 7 collaborative research projects using this technology, supported by 4 granting agencies, and involving 7 principal investigators in 3 countries.

Continued updates and enhancements of this technology will ensure its durability. Lack of familiarity with behavioral and computerized testing, the need for careful training of test administrators, and the lack of age-

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and country-specific norms are current constraints on adoption of this technology.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Presentation to Nonscientific Group:
Presentation of this research program and recent findings was made to students attending the InMed Summer Institute at the University of North Dakota, Grand Forks, ND, July 2001.
Media Coverage:
This research program, including study results, receives public exposure through frequent coverage by the popular press (local, regional and national newspapers, magazines, radio and television) and industry newsletters and magazines.

9. Scientific Publications:
01. Penland, J.G. "Zinc Nutrition affects cognition, psychosocial function and behavior throughout the life span". Invited address at the International Society for Trace Element Research in Humans. Quebec City, Canada. September 2001.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

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Accession: 0404369 Year: 01 Project Number: 5450-51520-012-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.2.2.2 25%
National Program(s): 107 100%

Title: BIOCHEMICAL, PHYSIOLOGICAL, AND NUTRITIONAL ROLES OF CERTAIN ULTRATRACE ELEMENTS

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Although emerging evidence indicates that certain mineral elements (e.g., arsenic, boron, nickel, silicon and vanadium) often called ultratrace elements can be involved in the prevention or amelioration of disease with nutritional roots, or for the enhancement of health and longevity, insufficient evidence exists to develop credible and data-supported dietary recommendations for these elements to assure health and well-being. Moreover, inadequate knowledge about health benefits of some ultratrace elements that are now only being discovered or defined (e.g., boron in bone and joint health) results in inappropriate and ineffectual reliance on other mineral nutrients (e.g., calcium) to provide these benefits. The lack of appropriate dietary recommendations for ultratrace elements allows charlatans for the purpose of financial gain to inappropriately promote these elements as supplements that can prevent some feared diseases such as cancer, osteoporosis, heart disease, and loss of cognitive function, or can enhance physical appearance. Thus, some of these elements are promoted (e.g., vanadium) in such a way that intakes detrimental to health may be occurring. Finally, the lack of recognition of health benefits provided by some ultratrace elements gives rise to risk assessments and toxicological standards by regulatory agencies that conflict with amounts that are known or predicted to be beneficial to health; this results in unnecessary efforts and expenditure of funds to reduce environmental exposure to amounts that in reality are not harmful, and may actually have health benefits. In summary, defined biochemical functions for ultratrace elements such as arsenic, boron, nickel, silicon and vanadium will establish these elements as essential nutrients, will allow the development of status indicators for the determination of dietary requirements to prevent any decline in health and well-being, and will allow for appropriate risk assessments and toxicological standards.

Animal and human experiments are and will be conducted to define the biochemical and physiological roles of various ultratrace elements including arsenic, boron, nickel, silicon and vanadium. The basic approach is to feed experimental animals and human volunteers diets that contain low, adequate, and/or luxuriant amounts of specific ultratrace elements and other selected nutrients and non-nutrients (postulated to affect the metabolism and utilization of specific ultratrace elements). The response of the animals and humans to the dietary manipulations will be ascertained

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by evaluating appropriate biochemical, physiological and anatomical variables. Biochemical and molecular biology methods will be used to define the specific essential role(s) of various ultratrace elements.

2. How serious is the problem? Why does it matter?

Dietary factors are associated with 5 of the 10 leading causes of death and with numerous chronic disorders; these include coronary heart disease, certain types of cancer, stroke, atherosclerosis, hypertension, osteoporosis and arthritis. Among those diseases that are linked strongly to diet, the cost of treatment and care in the United States exceeds $200 billion per year. Recognition that nutrition is important in health promotion and disease prevention has spawned a plethora of "health-enhancing foods" and supplements, now often called "functional foods" or "nutraceuticals" that represent an exploding market in the United States which exceeds $29 billion per year. Many of the health claims for these functional foods and nutraceuticals, however, have not been substantiated by basic research and feeding trials. Many of the health claims include the use of ultratrace elements because of some promising physiological or clinical finding (most often in an animal model or a special human situation) that has been extrapolated to their intakes having an influence on the susceptibility to, or severity of, one or more chronic diseases. Thus, there is a need to establish which foods, and their amounts, that will provide appropriate quantities of specific ultratrace elements of practical importance for the promotion of health and disease prevention or alleviation. Additionally, there is a need to determine safe intakes of specific ultratrace elements so that the setting of reasonable toxicological standards can be accomplished. Fulfilling these needs should result in policies and programs that will result in a healthier population, decrease the burden of chronic disease, enhance the quality of life, and diminish health care and environmental exposure protection expenditures.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research program relates to the National Program 107, Human Nutrition, and emphasizes the Program Component Performance Goal 3.1.1 - Human Nutrition Requirements. The challenge of this component is to identify essential nutrients, determine their effects on reproduction, development, function and longevity, and to provide information that will be used to develop standards to optimize human health, well-being, and genetic potential throughout the life cycle. All priority objectives, especially mechanism of action, biomarkers, function and performance, and nutrient interactions apply to the research program. Outcomes of the research will be knowledge that will facilitate the detection and prevention of biochemical, structural, physiological and psychological dysfunctions caused by the deficiency or imbalance of specific ultratrace

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elements, and will define requirements and safe intakes of specific ultratrace elements for health and well-being throughout the life cycle.

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishment during FY 2001:
Dietary boron in physiological amounts has anti-inflammatory properties in rats with adjuvant-induced arthritis; this suggests that dietary boron may influence the manifestations of human rheumatoid arthritis. Thus, in a collaborative study between C.D. Hunt of the Grand Forks Human Nutrition Research Center and D.S. Bradley of the University of North Dakota, genetically arthritic susceptible mice were immunized with heterologous type II bovine collagen to induce a polyarthritis that mirrors rheumatoid arthritis in its clinical, sereological, and histological manifestations. Mice supplemented with physiological amounts of dietary boron were resistant to induced arthritis, while mice deprived of dietary boron were highly susceptible to induced arthritis. These findings suggest that dietary boron, in physiological amounts, is an excellent nontoxic candidate for regulating inflammation in inflammatory autoimmune diseases such as rheumatoid arthritis.

B. Other Significant Accomplishment(s):
Although other laboratories have provided experimental evidence from animal models that boron is rapidly and nearly completely absorbed and excreted after ingestion, the rate and degree of absorption of boron from natural foods needs to be established in humans. At the Grand Forks Human Nutrition Research Center, a human subject was fed a single meal of B-10 enriched broccoli to facilitate the determination of boron metabolism characteristics by using isotope dilution methods. Urinary B-10 enrichment was detectable in the first post-prandial collection (0.5 hours), peaked at 2.5 hours, and concluded at 100 hours. This finding is the first to indicate that the form of boron in natural and usual food sources is highly bioavailable to humans.

Because physiological amounts of dietary boron can affect bone metabolism in several different ways, there is a need to establish whether the putative effect of some dietary minerals and vitamins on bone health should be ascribed to interactions between these dietary components and boron. For eight selected age-sex groups, dietary boron intake from 234 highly representative foods was compared to that of vitamins and other minerals from the same foods. It was found that all age-sex groups consumed, on a molar basis more boron than copper, manganese, or molybdenum; that boron intake correlated with calcium intake; and that diets with restricted boron intake typically restricted vitamin intake, especially ascorbic acid (vitamin C). The findings indicate that further research is needed to determine whether some of putative effects of dietary calcium and ascorbic acid on bone maintenance should be ascribed to boron, or whether the effects of these nutrients should be recognized as being influenced by

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dietary boron.

Nickel potentiates cyclic nucleotide-gated (CNG) cation channels; thus, there is a need to ascertain whether nickel deprivation adversely affects organs such as the kidney where CNG cation channels are important. Weanling rats were fed nickel-deficient or nickel-adequate diets containing normal or excessive sodium chloride (a stressor of kidney function and health) for nine weeks. Nickel deprivation impaired natriuresis (sodium excretion) after an oral sodium load, increased urinary N-acetyl-D-beta-glucosaminidase when sodium chloride was fed, and caused macroscopic and microscopic alterations in the kidney including smooth muscle layering in arcuate and interlobular arteries and protein casts in distal tubules. The findings indicate that nickel is a nutritionally important mineral element that is needed for optimal kidney function and health, and if lacking in the diet, contributes to the adverse effects of excessive salt (sodium chloride) intake.

Nickel potentiates the cyclic GMP-gated cation channel; thus, there is a need to ascertain whether nickel intake alters functions dependent upon these channels such as vision, smell and taste. Weanling rats were fed nickel-deficient or nickel-adequate diets for nine weeks then given tests to assess vision, smell and taste. Nickel deprivation altered the preference to saccharin taste and diminished darkness preference at 10 lux brightness. The findings suggest that nickel is essential for the function cyclic GMP-gated channel activity and thus is important in vision, smell and taste.

C. Significant Accomplishments/Activities that Support Special Target Populations.
None.

D. Progress Report.
None.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

New bridging CWU; all accomplishments listed above.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY 2002: The effects of boron deprivation and physiological amounts of boron on rheumatoid arthritis in human subjects will be determined with an emphasis on measuring variables that will indicate whether low boron nutriture, not uncommon in the United States, is a factor in the prevalence and severity of rheumatoid arthritis.

Experiments and tests will be performed to ascertain whether boron has a function that affects the functions of docosahexaenoic acid (DHA) thus

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resulting in altered vision and brain function, especially when dietary DHA and its precursors are marginal.

The impact of selenium deprivation on methionine metabolism will be further defined; the focus will be on the effect of selenium on homocysteine and the resulting impact on cardiovascular disease.

Through the use of isotopic arsenic, arsenic-dependent proteins from animals will be isolated and their relationship to methyl metabolism will be determined. Animal and cell culture experiments using graded concentrations of arsenic will be performed to compare the effects of physiological or nutritional amounts of arsenic with deficient or toxic amounts of arsenic on DNA methylation thus defining arsenic intakes that lead hypomethylation of DNA which is associated with an increased susceptibility to some forms of cancer.

A database of nickel concentration in foods will be compiled as an initial step in mining databases (i.e., NHANES) to determine whether there are correlations between nickel consumption and hypertension and cardiovascular health.

Ovariectomized rats (a model of osteoporosis) will be used to ascertain whether silicon deprivation results in increased bone loss through decreased bone formation during remodeling and growth because of detrimental changes in factors involved in the formation of the organic matrix in which calcification is initiated.

FY 2003: Studies will be initiated to determine boron-binding biomolecules relevant to boron absorption, transport, and excretion.

The effect of selenium status on nitrosothiol formation will be determined.

Experiments will be performed to establish whether arsenic deprivation enhances the susceptibility of experimental animals to chemically induced cancer. Specific tissues from animals fed diets containing deficient or adequate arsenic will be used in gene arrays to determine whether some specific genes involved in methionine metabolism are up or down regulated; differential changes will be used to establish the role of arsenic in methionine and methyl metabolism.

Databases such as NHANES will be gleaned to determine whether nickel consumption is associated with hypertension and cardiovascular health.

Experiments will be performed to test the hypothesis that an essential physiological function of nickel is the modulation of cyclic nucleotide gated channels activated by the binding of cGMP produced in response to atrial naturetic peptide (ANP) and nitric oxide (NO). Animals will be fed deficient and adequate nickel and various determinations will be made including the response to ANP, sodium nitroprusside, and a sodium chloride challenge test. Gene arrays will be used to ascertain whether nickel up or down regulates genes involved in cyclic nucleotide gated channel formation or function.

Cell culture studies will be performed to determine silicon metabolism and silicon utilization for bone maintenance and wound healing; this includes the use of gene arrays to ascertain whether genes for certain

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biomarkers of bone remodeling and collagen formation are up or down regulated with changes in silicon status.

FY 2004: Studies will be initiated to elucidate the induction mechanism whereby dietary boron increases the concentration of natural killer cells in the serum of animals models with experimental arthritis.

An experiment with human volunteers will be initiated to establish whether they respond to nickel deprivation in a manner similar to animals and thus establish nickel as nutritionally important. Status indicators developed from animal experiments in FY 2002 and FY 2003 will be used.

An experiment with human volunteers will be initiated to establish whether silicon is of practical importance for bone maintenance and thus a factor in the occurrence of osteoporosis. Status indicators developed from animal and cell culture experiments in FY 2002 and FY 2003 will be used.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Information about the nutritional or beneficial aspects of ultratrace elements as it becomes available is routinely transferred to a variety of customers. The customers include risk assessment groups through direct contact or organized meetings and workshops; the public through web pages of professional organizations and the Grand Forks Human Nutrition Research Center, and via the popular media; and other scientists through presentations at national and international meetings and professional publications.

Technology related to defining human dietary boron requirements and functions was described and discussed with industry representatives and plant boron nutrition scientists at a formal international workshop held in Bonn, Germany, July, 2001.

Information was transferred to the public through an article in the local newspaper (Grand Forks Herald) which also was placed on the Grand Forks Human Nutrition Research Center Home Page; this article was "Homocysteine, the new bad guy" by Eric Uthus.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Articles in the popular media often mention boron as a nutrient important for bone and joint health; this is based on findings from research done in this CWU. No attempt is made to keep a record of these articles.

9. Scientific Publications:

01. Yokoi, K., Lukaski, H.C., Uthus, E.O., Nielsen, F.H. Use of bioimpedance spectroscopy to estimate body water distribution in rats fed high dietary sulfur amino acids. Journal of Nutrition. 2001. v. 131. p. 1302-1308.

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National Program(s): 107 100%

Publications: (Continued)

02. Uthus, E.O. Arsenic deprivation affects homocysteine remethylation in rats fed homocysteine and choline. Proceedings of the North Dakota Academy of Science. 2001. v.55. p. 65. Topic presented by E.O. Uthus at the Annual North Dakota Academy of Science meeting. Bismarck, ND. April 2001.

03. Hunt, C.D., Idso, J.P. Dietary boron and erythritol and antigen injection interact to modify blood concentrations of NK cells and expression of CD45RC and CD4 and CD8a T cells in rats. The FASEB Journal. 2001. v. 15. pA1090. Presented by C.D. Hunt at the Experimental Biology 2001 meeting. Orlando, FL. March 2001.

04. Uthus, E.O., Davis, C.D., Yokoi, K. Selenium deprivation decreases the activity of liver betaine homocysteine methyltransferase in rats. The FASEB Journal. 2001. v.15. p.A969. Presented by E.O. Uthus at the Experimental Biology 2001 meeting. Orlando, FL. March 2001.

05. Yokoi, K., Uthus, E.O., Nielsen, F.H. Dietary sulfur amino acids and nickel deprivation affect the distribution of vitamin B6 vitamers in rats. The FASEB Journal. 2001. v. 15. p. A973. Presented by K. Yokoi at the Experimental Biology 2001 meeting. Orlando, FL. March 2001.

06. Yokoi, K., Uthus, E.O., Nielsen, F.H. Nickel deficiency induces renal damage and hypertension in rats which is augmented by sodium chloride. The FASEB Journal. 2001. v.15. p. A973. Presented by K. Yokoi at the Experimental Biology 2001 meeting. Orlando, FL. March 2001.

07. Nielsen, F.H., Uthus, E.O., Yokoi, K. Dietary nickel deprivation decreases sperm motility and evokes hypertension in rats. The FASEB Journal. 2001. v. 15. p. A972. Presented by F.H. Nielsen at the Experimental Biology 2001 meeting. Orlando, FL. March 2001.

08. Seaborn, C.D, Nielsen, F.H. Cysteine, silicon and their interaction affect bone composition in the rat. The FASEB Journal. 2001. v. 15. p. A972. Presented by C.D. Seaborn at the Experimental Biology 2001 meeting. Orlando, FL. March 2001.

09. Fohrman, S.K., Seaborn, C.D., Nielsen, F.H. Lead, silicon and their interaction affect bone composition. The FASEB Journal. 2001. v. 15. p. A972. Presented by S.K. Fohrman at the Experimental Biology 2001 meeting. Orlando, FL. March 2001.

10. Hunt, C.D. Boron-binding biomolecules: a key to understanding the beneficial physiologic effects of dietary boron from prokaryotes to humans. Boron 2001 International Workshop Book of Abstracts, Bonn, Germany. 2001. p. 26. Presented by C.D. Hunt at the Boron 2001 International Workshop. Bonn, Germany. July 2001.

11. Nielsen, F.H. The nutritional importance of boron throughout the life cycle of higher animals and humans. Boron 2001 International Workshop Book of Abstracts, Bonn, Germany. 2001. p. 33. Presented by F.H. Nielsen at the Boron 2001 International Workshop. Bonn, Germany. July 2001.

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Publications: (Continued)

12. Nielsen, F.H. The emergence of boron, nickel, silicon, vanadium and arsenic as elements of nutritional and pharmacological relevance. Abstract of presentation by F.H. Nielsen at First International Bio-Minerals Symposium: Trace Elements in Nutrition, Health and Disease. Salt Lake City, UT. April 2001.

13. Durick, K., Thiele, A., Cleland, H., Griffiths, M.M., Hunt, C.D., Bradley, D.S. Dietary boron prevents the onset of collagen-induced arthritis (CIA) in mice. Abstract of presentation at the Frank Low Research Day. University of North Dakota. April 2001.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

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Accession: 0401535 Year: 01 Project Number: 5450-51520-012-03 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 50% 5.2.2.2 50%
National Program(s): 107 100%

Title: THE NUTRITIONAL ROLE OF BORON IN THE INHIBITION OF SERINE PROTEASES

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? Y

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The cause of rheumatoid arthritis is unknown, a cure is unavailable, and the lifetime cost of rheumatoid arthritis for an individual patient can be as much as $250,000. Excessive inflammation leads to inflammatory disease (for example, rheumatoid arthritis). Certain boron compounds are potent in vitro inhibitors of several enzymes that regulate the normal inflammatory reaction. Therefore, the focus of this project is to identify inflammatory mediators that interact with boron. This approach will help establish the specific function of boron in humans and how dietary boron influences rheumatoid arthritis. Based on molecular structure, several classes of compounds predicted to interact with boron will be selected and assessed to determine the exact role of boron in regulation of the inflammatory response. The basic approach will be to determine the in vitro binding affinities of these compounds to boron. Subsequently, compounds with physiologically significant boron binding affinities will be investigated as potential indicators of boron status by in vivo measurement of these compounds after extraction from tissues of animals fed boron deficient diets.

2. How serious is the problem? Why does it matter?

Inflammatory diseases cost Americans billions of dollars yearly in treatment and loss of productivity. Rheumatoid arthritis in particular is a painful, chronic, recurrent, systemic inflammatory disease that affects 1-3 percent of Americans. Thus, prevention or significant amelioration of inflammatory diseases including rheumatoid arthritis by relatively simple dietary means, would have significant impact. There is a high probability that normal amounts of dietary boron will significantly ameliorate symptoms of rheumatoid arthritis in humans based on its known effect on cartilage development and maintenance and influence on the progression of experimental rheumatoid arthritis in animal model systems. Identification of biomolecules that interact directly with boron will serve to define borderline boron deficiency and develop indicators of boron deficiency.

3. How does it relate to the National Program(s) and National Program Component(s)?

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The research relates to the National Program 107, Human Nutrition (100%), specifically, the National Program Component of Human Nutrition Requirements: "determine requirements for nutrients and other food components of children, pregnant and lactating women, adults, and elderly of diverse racial and ethnic backgrounds." The research relates to the "Mechanism of Action" objective within this component: "Identify and fully characterize mechanisms of action for beneficial effects of known nutrients and other potentially beneficial dietary chemicals; measure the size of the effects associated with specific amounts of the chemical or nutrient component in question."

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishment during FY 2000 year:
See Progress Report.

B. Other Significant Accomplishment(s):
None.

C. Significant Accomplishments/Activities that Support Special Target Populations:
None.

D. Progress Report:
This report serves to document research conducted under a trust fund cooperative agreement between ARS and U.S. Borax, Inc. Additional details of research can be found in the report for the parent project 5450-51520-012-00D Biochemical, Physiological, and Nutritional Roles of Certain Ultratrace Elements. Identification of biomolecules that interact directly with boron will serve to define borderline boron deficiency and develop indicators of boron deficiency. A new capillary electrophoresis method developed in this laboratory was used to demonstrate the direct binding of boron to biomolecules of considerable physiological importance. Physiological amounts of boron were found to bind to S-adenosylmethionine, the predominant methyl donor in biological methylations and a versatile co-factor in a variety of physiological processes. These findings suggest that boron, a natural dietary component, may be important in the regulation of substrate methylation in biochemical pathways. Determining whether human cells actively take up boron from the extracellular fluid would provide additional evidence for an essential role for boron in humans. A new methodology that uses flow cytometry was developed to accurately determine the amount of media taken up by human cells cultured in growth media. The subsequent finding that a human cell line grown in a normal culture medium sequesters boron provides additional indirect evidence for an essential role for boron in animals and humans.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

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An in vitro model system was developed and implemented to determine the direct binding of boron to biomolecules. The method utilizes capillary electrophoresis technology and allows for the discrete separation and identification of biomolecules that bind to boron and also allows for discrimination of binding interactions. When molecules are separated by capillary electrophoresis in the absence or presence of boron, the degree of boron binding is indicated as a change in migration time of the molecule through the solution. An increase in migration time indicates increased boron binding. Various biomolecules can be compared for their ability to bind boron. The information may be used to determine the importance the exact biological role of boron in humans and the importance of that role in the inflammatory response.

The finding that cells isolated from a mouse monocyte-macrophage cell line and grown in a normal culture medium internalize boron provides additional indirect evidence for an essential role for boron in animals and humans.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY 2002 - New methodology will be developed and implemented to identify the large number of biomolecules, isolated from whole blood, that are predicted to bind to physiological amounts of boron. FY 2003 - Experiments will be conducted to determine whether tissue concentrations of biomolecules that bind to boron and are known to have a role in inflammation are influenced by boron-deprivation in an experimental rheumatoid arthritis animal model. FY 2004 - Experiments will be conducted to determine the physiologic amount of dietary boron needed to provide maximal amelioration of experimental rheumatoid arthritis and optimal blood concentrations of biomolecules previously determined to bind to boron.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Technology related to the new method developed in this laboratory to detect boron-binding biomolecules was described and discussed to plant boron nutrition scientists and industry representatives at a formal international meeting held in Bonn, Germany, July 24, 2001.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

None.

9. Scientific Publications:

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401535 Year: 01 Project Number: 5450-51520-012-03 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 50% 5.2.2.2 50%
National Program(s): 107 100%

Publications: (Continued)
01. Publications from this project are included in the report for the parent project 5450-51520-012-00D Biochemical, Physiological, and Nutritional Roles of Certain Ultratrace Elements.

Approved: HENRY C LUKASKI Date: 09/01
Title: ACTING CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0402077 Year: 01 Project Number: 5450-51520-012-04 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 80% 5.2.2.2 20%
National Program(s): 107 100%

Title: DETERMINATION OF THE ESSENTIALITY OF NICKEL

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? Y

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:
1. What major problem or issue is being resolved and how are you resolving it?

2. How serious is the problem? Why does it matter?

3. How does it relate to the National Program(s) and National Program Component(s)?

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishments during FY 2001:

B. Other Significant Accomplishments:

C. Significant Accomplishments/Activities that Support Specific Target Populations:

D. Progress Report:
This report serves to document research conducted under a trust received by ARS from the Nickel Producers Environmental Research Association (NIPERA). Funds were used to support a post doctorate (K. Yokoi) and were depleted by March 3, 2001. Additional details of research can be found in the report for the parent bridging CRIS 5450-51520-012-00D, Biochemical, physiological, and nutritional roles of certain ultratrace elements, and the predecessor CRIS 5450-51520-011-00D with the same title. During the final time period supported by the trust funds, further support was obtained for the hypothesis that nickel is involved in the function of cyclic GMP gated cation channels that affect vision, olfaction, renal function, sodium metabolism, blood pressure control, sperm physiology, and male sexual function.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0402077 Year: 01 Project Number: 5450-51520-012-04 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 80% 5.2.2.2 20%
National Program(s): 107 100%

6. What do you expect to accomplish, year by year, over the next 3 years?

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

9. Scientific Publications:

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404371 Year: 01 Project Number: 5450-51530-006-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.3.1.1 25%
National Program(s): 107 100%

Title: MINERAL ELEMENTS, PHYSIOLOGICAL FUNCTION AND PERFORMANCE AND BODY COMPOSITION

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? N

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:
1. What major problem or issue is being resolved and how are you resolving it?

Recommendations for the dietary intake of mineral elements, with an emphasis on copper, chromium, iron, magnesium, and zinc, based on the promotion of health and optimal biological function are generally lacking. Studies designed to examine the effects of graded dietary intakes of mineral elements on physiological function are needed to ascertain appropriate amounts of mineral elements in the diet to maintain health and to facilitate the attainment of genetic potential of biological functions. One consideration in delineating appropriate dietary mineral intakes is assessment of food-borne factors that affect the absorption and utilization of dietary minerals. Also, the use of environmental stressors (i.e., controlled exercise and cold/hot temperatures) is another factor used to determine mineral element needs of physically active people.

Iron deficiency is the single most prevalent nutritional deficiency in the world. Attempts to fortify food products have been only partially successful in ameliorating this pervasive nutritional problem among women. In parallel with the incidence of iron deficiency, there has been an increase of polyunsaturated fat intake. Studies indicate that the type of dietary fat affects iron absorption and utilization, specifically non-heme iron. Polyunsaturated fat reduces and saturated fat, specifically stearic acid, promotes iron utilization. Because stearic acid is neutral to serum cholesterol and lipoprotein cholesterol concentrations, it offers practical benefits in ameliorating iron deficiency in animals and humans.

Routine assessment of human body composition is hampered by the lack of sensitive and specific methods to measure bone mass and quality, fat and muscle in national nutritional surveys and repeatedly in response to medical and nutritional interventions.

Studies are conducted in animals and humans. Graded intakes of dietary zinc and copper are fed and physiological functions are monitored to delineate intake amounts that affect physiological function with an emphasis on energy utilization, cardiorespiratory function, work performance, and heat production. Studies of human volunteers fed whole food diets low in chromium and supplemented with specific chromium compounds and other compounds hypothesized to influence the absorption and utilization of chromium and other minerals are conducted. Other studies are undertaken in which animals are fed a diet low in iron, then given diets containing varied amounts of iron (low and adequate) and different types

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404371 Year: 01 Project Number: 5450-51530-006-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.3.1.1 25%
National Program(s): 107 100%

(saturated and polyunsaturated) of dietary fat. Changes in iron status and hematology, as well as changes in bone mineral content, are determined.

Studies are undertaken in animals and humans to develop and validate methods for use in assessing nutritional status and effects of nutritional intervention on bone and soft tissue composition.

2. How serious is the problem? Why does it matter?

There is considerable debate regarding the amount of dietary copper, chromium, magnesium, and zinc required for health maintenance and optimal biological function. Previous approaches focused on relatively insensitive measures of nutritional adequacy (chemical balance). By relating dietary mineral intakes to measurements of biological function, such as energy utilization, heart rate and blood pressure, work production, heat generation, and glucose and lipid metabolism, suggestions for dietary mineral intakes are made in reference to quality and quantity of life. Much of this research is requested by physically active individuals who seek to optimize physiological function without the use of dietary supplements and health managers who seek to minimize health care costs of the American public.

Development and validation of sensitive and specific methods to assess human body composition remain fundamental needs for nutritional assessment of healthy persons of all ages and backgrounds as well as individuals with chronic disease and medical intervention. Moreover, these methods are required for establishment of national distributions or norms for body fat and muscle for use as references in nutritional interventions to promote health.

3. How does it relate to the National Program(s) and National Program Component(s)?

This work relates to National Program 107, Human Nutrition(100%). The specific Program Components include Nutrient Requirements with the objectives of defining Biomarkers of Marginal or Borderline Deficiencies, Mechanisms of Action of Mineral Elements, Effects of Environmental and Lifestyle Factors, and Achievement of Optimal Function and Performance.

This research will acquire information about the effects of graded trace element deficiencies, emphasizing copper, chromium, iron, magnesium, and zinc, on biochemical measurements of mineral nutritional status and physiological functions. This information will facilitate the detection of marginal mineral deficiencies and define dietary requirements of these mineral elements for the development and maintenance of health and optimal function throughout the life cycle. The research will provide needed information that can be used to assess the risk of chronic diseases and impairments in subtle physiological functions that arise from mild and moderate mineral element deficiencies. In addition, new methods for routine assessment of human body composition will be developed and validated for

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404371 Year: 01 Project Number: 5450-51530-006-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.3.1.1 25%
National Program(s): 107 100%

non invasive assessment of human nutritional status, particularly conditions that exhibit weight change.

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishments during FY 2001:
Routine assessment of copper nutritional status is hampered by the lack of availability of a sensitive and specific biochemical marker. In collaboration with Dr. T. Oury (University of Pittsburgh School of Medicine), we compared the serum extracellular copper-zinc superoxide dismutase (SOD3) activity and protein concentration determined by two methods in rats fed graded amounts of dietary copper. We found that the method that uses xanthine oxidase compared to pyrogallol yielded results that were highly sensitive and responsive to graded copper deprivation. Impact: These initial findings strongly suggest that SOD3 activity is a novel and sensitive marker of copper status and may be a useful indicator of copper status in humans.

B. Other Significant Accomplishments:
None.

C. Significant Accomplishments/Activities that Support Specific Target Populations:
Placed into service a Mobile Nutrition Research Laboratory to conduct nutrition studies with American Indians living in the Northern Plains Area, with rural populations and other specific targeted groups who could not otherwise participate in nutrition studies (e.g., children, adolescents and the elderly). This mobile laboratory will support research studies to determine in these at-risk populations the relationships between mineral nutrition and chronic diseases such as obesity, diabetes and dementia, and between mineral nutrition and physiological and behavioral function. Nutritional, physical activity and behavioral interventions that utilize develop culturally-sensitive approaches and methods will be planned and initiated in these at-risk populations to reduce the probability of certain chronic diseases including obesity, diabetes, cardiovascular disease, and dementia.

D. Progress Report:
None.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

This is a new project replacing the previous project, Mineral Elements, Physiological Function & Performance and Body Composition (CRIS No. 5450-51530-003-00D), terminated 03-03-01.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404371 Year: 01 Project Number: 5450-51530-006-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.3.1.1 25%
National Program(s): 107 100%

6. What do you expect to accomplish, year by year, over the next 3 years?

FY 2002: Determine the individual and combined effects of graded dietary zinc and copper on activities of superoxide dismutase enzymes as markers of zinc and copper status relative to traditional biochemical markers of zinc and copper nutriture of rats. Incorporate measures of extracelluar superoxide dismutase protein to assess the specific activity of this enzyme as a marker of copper or zinc status and evaluate if expression of message of this protein is affected by dietary minerals. Findings will provide a basis for planning future human studies to delineate mineral requirement to control oxidative damage during physical training.

FY 2002: Ascertain the effects of graded dietary copper on resting energy metabolism and thermogenesis in the cold in rats. Test the hypothesis that copper restriction impairs the induction and expression of uncoupling proteins. Determine the effects of copper depletion independently of anemia on the metabolic and hormonal perturbation in energy metabolism of copper-deficient rats. This study will identify new physiologic role(s) of copper in regulating energy expenditure.

FY 2002: Delineate the effects of dietary zinc and exercise on the induction and expression of zinc-containing enzymes involved in energy production in rodents. By using graded dietary zinc and different types of exercise training (endurance and resistance), this study will determine if limiting zinc intake has adverse effects on ability to improve physical performance by limiting the production and activity of specific zinc-containing enzymes (e.g., carbonic anhydrase and lactate dehydrogenase). Findings will provide a practical basis for assessing zinc requirements of physically active persons.

FY 2002: Plan and initiate a field study to determine the effects of zinc intake provided in food on functional parameters including growth, body composition, cognitive function, and physical fitness of adolescents. A new mobile research unit laboratory will be used to make all assessments. Findings will be useful in determining zinc intakes to promote optimal physical and behavioral development of adolescents at-risk for zinc deficiency because of rapid growth.

FY 2003: Plan and initiate a health and nutrition survey of Native American tribes in North Dakota to determine relationships among mineral nutritional status and health with an emphasis on obesity, diabetes and cardiovascular disease. Results will provide a basis for planning future mineral supplementation trials to test the hypothesis that remediation of mineral deficiency will decrease incidence of some chronic diseases.

FY 2003: Design and implement a study of older adults to discern the impact of antihypertensive medications on zinc and magnesium status and assess the impact of supplemental zinc and magnesium, either as supplements or food fortification, on muscle function and body cell mass. Results will provide needed information regarding the mineral needs of older adults to preserve muscle mass and physiological and mental capacities.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404371 Year: 01 Project Number: 5450-51530-006-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.3.1.1 25%
National Program(s): 107 100%

FY 2003: Conceive, plan and initiate a study of the biochemical mechanism(s) of trivalent chromium on intracellular signaling pathways associated with insulin and glucose metabolism. Results will provide the basis for planning human supplementation trials of type 2 diabetics to delineate the effects of supplemental trivalent chromium on glycemic control.

FY 2004: Plan and initiate a community feeding study to determine relationships between zinc intake, strength gain and body composition change of adults during resistance training. In collaboration with the University of North Dakota, healthy adults will be randomized and fed graded dietary zinc and participate in controlled resistance training. Outcome measures will include strength gain, muscle mass accretion, zinc status, and anabolic hormone responses. Findings will contribute new information about adaptation of zinc metabolism and status in response to strength-building.

FY 2004: Conceive, plan and initiate a study to elucidate the effects of different weight loss programs and interventions on mineral nutrition status, weight loss and maintenance. Obese adults and adolescents will participate in dietary, behavioral and physical interventions for a 3 month period, then move to a self-continued program for another 6 months. Outcome will include biochemical measures of mineral nutritional status, preservation of muscle and bone and maintenance of weight loss.

FY 2004: Plan and initiate a study in rats to determine the effects of graded dietary copper on reactive oxygen and nitrogen species on angiogenesis and skeletal muscle apoptosis as beneficial adaptive responses to exercise. Results will provide needed information to plan human study of copper-effects on physiological adaptation to aerobic exercise.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Information about the basic and applied aspects of mineral nutrition as it becomes available is routinely transferred to a variety of customers. The customers include other federal agencies and national and international sport groups through direct contact or organized meetings and conferences; the public through web pages of professional organizations and the Grand Forks Human Nutrition Research Center, and via popular media; and other scientists through presentations at national and international meetings and professional publications. Results from studies are published in peer-reviewed scientific journals. Upon publication, the methodology, data, and interpretations of the data are immediately available to scientists and other interested parties. In addition, experimental results and educational information pertinent to public interests are made available through the local newspaper, The Grand Forks Herald, and statewide through the Interactive Video Network of the North Dakota State University Service

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404371 Year: 01 Project Number: 5450-51530-006-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.3.1.1 25%
National Program(s): 107 100%

Continuing Education Program.
Some examples of technology transfer include articles in lay public publications and televison interviews: "Minerals and Energy" in Women's World, "Chromium and Weight Loss" on FOX Channel 8 in Cleveland, OH, and "Update on Chromium and vanadium effects on diabetes" in Nutrition Action Newsletter, May-June 2001.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

University of North Dakota, Strength and Conditioning Seminar, April 6, 2001, Grand Forks, ND. Lecture - Supplements - An NCAA and International Perspective.
36th Annual Convention of the National High School Athletic Coaches Association, June 24, 2001, Fargo, ND. Lecture - Nutritional Ergogenic Aids in Sport.
International Scientific Conference on Nutrition and Athletic Performance, August 7, 2001, Edmonton, Alberta, Canada. Lecture - Magnesium, Zinc and Chromium Nutrition and Athletic Performance.

9. Scientific Publications:

01. Yokoi, K., Lukaski, H.C,. Uthus, E.O., Nielsen, F.H. Use of bioimpedance spectroscopy to estimate body water distribution in rats fed high dietary sulfur amino acids. Journal of Nutrition. 2001. v. 131. p. 1302-1308.

02. Lukaski, H.C., Bolonchuk, W.W., Klevay, L.M., Milne, D.B., Sandstead, H.H. Interaction of dietary fat, mineral status and performance of endurance athletes. International Journal of Sports Nutrition. 2001. v. 11. p. 186-198.

03. Lukaski, H.C., Marchello, M.J., Hall, C.B., Siders, W.A. Validity of dual x-ray absorptiometry (DXA) to assess body composition of rats exposed to various stressors. Nutrition. 2001. v. 17. p. 607-613.

04. Lukaski, H.C. Body mass index, bioelectrical impedance, and body composition. Nutrition. 2001. v. 17. p. 55-56. (Invited Editorial).

05. Lukaski, H.C. Body composition distribution with age - growth charts for adults? Nutrition. 2001. v. 17. p. 675. (Invited Editorial).

06. Lukaski, H.C., Johnson, P.E. Dietary copper, independent of anemia, impairs cardiorespiratory function and muscle copper kinetics in men. The FASEB Journal. 2001. v. 15. p. A416. Presented by H.C. Lukaski at the Experimental Biology 2001 meeting. Orlando, FL. March-April 2001.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0404371 Year: 01 Project Number: 5450-51530-006-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.3.1.1 25%
National Program(s): 107 100%

Publications: (Continued)

07. Hill, K., Lukaski, H., Roughead, F. Low copper intake reduces serum IGF-1 and bone strength. Proceedings of the 9th Annual Experimental Program to Stimulate Competitive Research. 2001. Abstract #53. p. 15.

08. Lukaski, H.C. "Adverse effects of dietary zinc restriction on cardiorespiratory function during exercise in men." Presented at the International Society for Trace Element Research in Humans. Quebec City, Canada. September 2001.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

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INTERIM AND FINAL PROGRESS REPORTS

OF

TERMINATED CRIS WORK UNITS

MINERAL NUTRIENT REQUIREMENTS

MANAGEMENT UNIT

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149753 Year: 01 Project Number: 5450-51000-012-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Title: EFFECTS OF COPPER DEPLETION ON CARDIOVASCULAR FUNCTION AND METABOLISM

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? Y

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Diets in the U.S. frequently are low in copper in comparison to dietary standards set by the Food and Nutrition Board of the National Academy of Sciences; these diets resemble the low copper diets that have produced abnormal electrocardiograms, increased cholesterol in blood, impaired metabolism of sugar, and poor control of blood pressure in men and women in controlled experiments. Diets low in copper may contribute to human illness (See Question 2).

The approach to the problem is to identify new biochemistry and physiology of copper with animal experiments to provide functional biomarkers useful in dietary experiments with human volunteers and in community studies. The experiments will identify mechanisms by which adequate dietary copper produces beneficial effects, will identify new effects of human diets low in copper and will contribute to the establishment of national dietary standards. People with biomarkers suggestive of low copper intakes will be supplemented and their responses evaluated.

2. How serious is the problem? Why does it matter?

The major signs of copper deficiency found in depleted men and women and deficient animals resemble the most common characteristics that can predict risk of ischemic heart disease in people. Nearly 80 anatomical, chemical and physiological similarities between animals deficient in copper and people with ischemic heart disease have been identified. It seems likely that the low copper diet common in the U.S. contributes to this disease which is the leading cause of death in the U.S., 480,000 deaths annually. The cost of medical care for this illness is more than $5 billion per year which does not include effects of sorrow, time lost from work or annual cost of prevention (at least $1000 per person). Proper selection of foods may yield diets that meet the

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149753 Year: 01 Project Number: 5450-51000-012-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

standards necessary to both prevent illness and decrease expense. This work is relevant to dietitians, food companies, physicians, producers of grain, legumes, nuts and other foods high in copper, public health planners, and teachers of nutrition.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research program fits into the National Program 107, Human Nutrition and Performance Goal 3.1.1. Human nutrition requirements. Emphasis is on biomarkers, mechanisms of action, nutrient interactions and functions as related to healthy hearts and blood vessels to optimize longevity, to decrease disease incidence and to improve productivity. Cooperative studies are in progress with Loma Linda University and the Health Research and Studies Center to evaluate the effects of diets high in complex foods on biomarkers of trace element status and with the Medical College of Ohio to clarify the mechanisms by which copper deficiency alters electrocardiograms and heart function.

4. What were the most significant accomplishments this past year?

A. Dehydroepiandrosterone, commonly known as DHEA, is a hormone that decreases as people age. Higher amounts of DHEA may protect against heart attacks. Rats deficient in copper have half the normal amount of DHEA in blood serum. It may be both cheaper and safer to eat a diet high in copper or take copper supplements than to take DHEA supplements (which are readily available) to obtain the benefits of the hormone.

B. Both pets and animals in zoos often become deficient in copper from eating pennies because recently minted pennies contain very little copper and are so high in zinc that they interfere with copper utilization. If these unfortunate animals are treated with copper, they are more likely to survive. Examination of the hearts and bones of those animals that die may provide insights to human heart disease and the gradual loss of bone (osteoporosis) that occurs with aging.

Rats fed a diet marginal, but not deficient in copper, become deficient in copper when fed high dietary iron. As the U.S. diet often is low in copper, people with iron overload may benefit from copper supplements as may women who take iron supplements during pregnancy.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149753 Year: 01 Project Number: 5450-51000-012-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

A disparity between actual copper intakes and both copper requirements and dietary recommendations has been recognized from the publication by this author of dietary data pooled from 10 research groups in four countries to reveal 60% of daily diets contain less than 1.5 mg of copper and one third contain less than 1 mg. These results refute earlier nutritional beliefs that "adults generally consume 2 to 5 mg of copper daily."

High blood pressure from copper deficiency may be explained partially by the finding of impaired defense against damage by oxygen such as cholesterol oxidation as indicated by findings from copper-deficient animals of an accumulation of a lipid oxidation biomarker called isoprostane and a related impaired ability to relax blood vessels because of the destruction of nitric oxide which is a naturally occurring relaxing agent in blood vessels.

Superoxide dismutases are some of the enzymes that defend us against oxidative damage. The dismutase that depends on adequate dietary copper is impaired in copper deficiency, where, however, genetic control of the dismutase that depends on manganese is improved. This improvement generally is insufficient to eliminate the oxidative damage, and thus pathology caused by an inadequate intake of copper most likely partially involves an impaired defense against damage caused by reactive oxygen species.

All of these findings are related to the mechanisms by which the Western diet gradually damages arteries and hearts leading to ischemic heart disease (see Question 2). Improving diets low in copper by selection of foods high in copper and by decreasing intake of foods low in copper is likely to have great benefits to both health and to decreasing the vast annual expenditures on medical care in middle and old age (see Question 2). Regular consumption of diets adequate in copper may lengthen life and assist in attaining a healthier old age. The ARS Food Pyramid is a useful guide.

6. What do you expect to accomplish, year by year, over the next 3 years?

This project was discontinued on 3/28/01. Please see the report for project 5450-51000-026-00D for expectations of future accomplishments in this area.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149753 Year: 01 Project Number: 5450-51000-012-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The United States Pharmacopeial Convention (USP) provides standards to the Food and Drug Administration in efficacy, quality and safety, etc., of prescription medicines. Dr. Klevay is a member of the USP Bioavailability and Nutrient Absorption Expert Committee which writes approximately a half dozen nutritional monographs each year. These are incorporated into annually revised volumes published by the USP being immediately available to physicians and pharmacists and other regulatory agencies such as state medical boards.

Information was transferred to the public through articles in the local newspaper (Grand Forks Herald) which also were placed on the Grand Forks Human Nutrition Research Center Home Page; one article was "Ignored copper receives new RDA status" by Leslie M. Klevay.

L.M. Klevay organized the XIV Annual Copper Cardiovascular Cognoscenti dinner and the Ischemic Heart Disease and Malnutrition Workshop at Experimental Biology 2001 Meeting, Orlando, FL.

Several inquiries by individual citizens about brain copper, copper and environmental cancer, copper and heart disease, copper deficiency, dietary copper, fat and cholesterol and iron fortification were answered.

Several inquiries by institutional representatives about ARS priorities, copper in the elderly, hair analysis in nutritional assessment were answered.

Several scientific articles by Dr. Klevay were cited in the recent publication Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc available on the internet from the National Academy of Sciences.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Heart disease and malnutrition was presented at the Grand Forks Rotary Club.

9. Scientific Publications:

01. Klevay, L.M., Christopherson, D.M., Shuler, T.R. Variability of multiple nutritional elements in hair of one man over two decades. Roussel, A.M., Anderson, R.A., Favrier, A.E., editors. Kluwer Academic/Plenum Publishers, New York, NY. Proc. 10th International Symposium on Trace Elements in Man and Animals (TEMA-10). 2000. p. 339.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149753 Year: 01 Project Number: 5450-51000-012-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Publications: (Continued)

02. Klevay, L.M., Christopherson, D.M. Copper deficiency halves serum dehydroepiandrosterone in rats. Journal of Trace Elements in Medicine and Biology. 2000. v. 14. p. 143-145.

03. Klevay, L.M. Letter to the Editor. Journal of Zoo and Wildlife Medicine. 2000. v. 31. p. 289-290.

04. Lukaski, H.C., Bolonchuk, W.W., Klevay, L.M., Milne, D.B., Sandstead, H.H. Interactions among dietary fat, mineral status, and performance of endurance athletes: A case study. International Journal of Sport Nutrition and Exercise Metabolism. 2001. v. 11. p. 186-198.

05. Klevay, L.M. Iron overload can induce mild copper deficiency. Journal of Trace Elements in Medicine and Biology. 2001. v. 14. p. 237-240.

06. Klevay, L.M., Christopherson, D.M. Copper deficiency decreases plasma testosterone (T) in rats. Federation of American Societies for Experimental Biology Journal. 2001. v. 15. p. A272.

07. Klevay, L.M. Impaired oxidative defense in ischemic heart disease from diets low in copper? Journal of Molecular and Cellular Cardiology. 2001. v. 33. p. A59.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400377 Year: 01 Project Number: 5450-51000-020-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: HOMEOSTASIS AND BIOAVAILABILITY OF TRACE ELEMENTS IN HUMANS AND ANIMALS

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? Y

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:
1. What major problem or issue is being resolved and how are you resolving it?

The focus of our laboratory is determination of the health effects of consumption of dietary manganese and selenium. Both nutrients are essential, but they also are toxic at higher intakes. Our research is attempting to define the optimal intakes of these nutrients that result in maximal health benefits but do not result in toxicity.
A. Manganese is an essential element, but its practical nutritional importance has not been ascertained, nor has a safe upper limit of intake been established. Knowledge of safe intakes are needed because of recent speculation that high dietary intakes could have detrimental effects on brain function and behavior. Whether manganese ingested through the diet results in manganese accumulation and predisposition to toxicity, and factors that affect these processes need to be determined.

Human volunteers and animals will be used to determine factors affecting manganese absorption and retention in the body and body tissues. Interactions that may affect manganese metabolism include interactions with other trace elements, food and non-food components of plants and fat composition of animals. Studies will determine whether interactions with iron may affect both iron and manganese uptake and retention. Studies with magnesium deficient animals will determine whether diets high in manganese result in increased risk of magnesium-deficiency heart disease.
B. Supplemental selenium is being consumed by many as a prophylaxis against certain cancers, and for its antioxidant potential. These benefits of selenium depend on the chemical form consumed and different foods have different amounts and chemical forms of selenium. Our laboratory is determining the optimal forms and amounts of food forms of selenium for persons desiring to consume supplemental selenium. We are taking an integrated approach and are examining how selenium incorporates into the food, how food preparation affects that selenium, the basic metabolism of selenium from these foods (determined in laboratory animals) and how humans respond to

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400377 Year: 01 Project Number: 5450-51000-020-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

supplementation with these foods.

The efficacy of food sources of selenium for cancer prevention will be determined in rats injected with a carcinogen and fed diets containing selenium supplied as high-selenium meat, wheat or broccoli. To determine the ability of high-selenium foods to induce selenoprotein synthesis, animals and cultured cells will be supplied radioactive selenium incorporated into meat, wheat or broccoli. Proteins will be chemically separated and examined for the presence of radioactive selenium. The health benefits of these foods to humans will be determined in human supplementation studies conducted in selenium-adequate and selenium-deficient areas.

2. How serious is the problem? Why does it matter?

Studies of manganese intake and retention are important because excessive intakes of manganese result in toxicity symptoms that resemble Parkinson's disease. Recently, it has been suggested that the amounts of manganese consumed through food may be harmful and that efforts should be made to remove it from the food supply. There are little data to support these assertions, but likewise there are little data to refute them. This research will be of value to physicians, health professionals and regulatory professionals who will make decisions regarding the safety and adequacy of manganese in the North American diet.

The studies of food forms of selenium are important because selenium supplementation is a nutritional issue. Most nutritional professionals agree that the best way to consume nutrients is through the food supply, but presently there are no guidelines regarding the best food forms of selenium. Demonstrating that selenium in a food can produce health benefits similar to dietary supplements will benefit the consumer, as well as producers who may use the information to increase the marketability and/or profitability of their product. Studies of food forms of selenium are also important because such studies could lead to the production of specialty crops and/or food products that are sold for their enhanced selenium content.

3. How does it relate to the National Program(s) and National Program Component(s)?

These studies relate to National Program 107, Human Nutrition. This work is related to specific objectives of Bioavailability of Nutrients and Food Components, Health Promoting Properties of Plant and Animal Foods and to Nutrient

ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0400377 Year: 01 Project Number: 5450-51000-020-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Requirements.

4. What were the most significant accomplishments this past year?

A. Concerns about the possibility of either manganese toxicity or deficiency in free-living humans consuming mixed western diets, and the paucity of well-controlled human studies addressing these questions, were the impetus for a comprehensive human study that examined health effects of consuming diets made as low or high in manganese as practically possible. Human volunteers were fed less than 1 or 20 milligrams of manganese a day for sixty days, and clinical, neurological and physiological variables were closely monitored. This study was initiated a year previous but has only been completed in the past year. Manganese fed at the maximum or lowest amount practical in a mixed Western diet affected manganese retention but did not have any deleterious health consequences to healthy young women. The results indicate that, in the absence of exacerbating circumstances and/or dietary interactions, there is no practical reason to fear for human manganese deficiency or toxicity.

Studies with animals have demonstrated that high manganese combined with a deficiency of magnesium results in increased risk of heart disease, and that this risk may be mediated by manganese substitution for magnesium in magnesium dependent proteins.

B. Because of the reports of the anti-cancer properties of selenium, many persons would like to consume more selenium through their diet, but different foods contain different forms of selenium, and the optimal food form(s) of selenium have not been identified. We have identified farming methods that result in the production of high-selenium wheat, broccoli and beef. We have determined geographical and geochemical factors that affect accumulation of selenium in these foods and have determined the relative effectiveness of these foods for preventing colon cancer. Determined the relative effectiveness of selenium from these foods for incorporation into selenium proteins.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Demonstrated that supplemental selenium improves the mood of healthy young men. This effect was seen in selenium-adequate and selenium-deficient men. This research adds to the

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National Program(s): 107 100%

increasing number of studies showing that supplemental intakes of selenium improve health and well-being.

Demonstrated in rats that selenium from high-selenium broccoli was retained and distributed differently than selenium from salts such as selenite and selenate. The difference in the distribution of selenium allows selenium from broccoli to be less toxic to rats than other forms of selenium, and also to have greater anti-cancer activity. This research demonstrates that all selenium-containing foods are not equal, and that health professionals need to consider the source of selenium before recommending increased intakes.

Developed a process to label broccoli with stable isotopes of selenium, and demonstrated in healthy young men that selenium from broccoli did not accumulate to the extent that selenium from salts did. This study demonstrated in humans that selenium from broccoli is less toxic than inorganic chemical forms of selenium.

Demonstrated that selenium from high-selenium broccoli was more effective than salt or amino acid forms of selenium for prevention of colon cancer. Found that high-selenium broccoli sprouts were as efficacious as broccoli florets for prevention of colon cancer.

Described, by using cultured cells, how the cell accumulates manganese and demonstrated that manganese is quickly moved in a direction that results in excretion into the gut, whereas movement in the direction of absorption is slow. This finding shows that animals have developed complex mechanisms to keep manganese from accumulating in the body, and that in the absence of exacerbating factors and interactions, manganese toxicity is probably not of practical concern.

Demonstrated in humans that very little manganese is absorbed, and that absorption is strongly associated with the iron status of an individual. This finding demonstrates that interactions of other nutrients with manganese may potentiate manganese absorption. Thus, future studies of possible manganese toxicity should concentrate on possible adverse interactions.

Demonstrated that magnesium-deficient pigs that are fed moderately high amounts of manganese are at an increased risk of sudden death by heart disease. This finding also demonstrates that interactions of other nutrients with manganese may potentiate manganese absorption/retention, and that studies of possible manganese toxicity should concentrate on adverse interactions.

6. What do you expect to accomplish, year by year, over the next 3 years?

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Report of Progress (AD-421)

Accession: 0400377 Year: 01 Project Number: 5450-51000-020-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

This project has been discontinued. Please see the report for project 5450-51000-029-00D for expectations of future accomplishments in this area.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Research was transferred to customers by a variety of mechanisms including:

Human manganese studies were used to formulate manganese Dietary Reference Intakes released by the National Academy of Science.

Presented information regarding the anti-cancer effects of selenium from broccoli at the International Selenium Symposium in Venice, Italy.

A Cooperative Research and Development Agreement was developed with General Mills to assist in the development and human testing of high-selenium wheat products.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Technology has been transferred through a variety of activities by writing articles for the local newspaper as follows: Do you sell a commodity or food? Grand Forks Herald, 7/26/00.

9. Scientific Publications:

01. Finley, J.W., Davis, C.D., Feng, Y. Selenium from high selenium broccoli protects rats from colon cancer. Journal of Nutrition. 2000. v. 130. p. 2384-2389.

02. Finley, J.W. Use of Isotopes for Studies with Manganese, Chromium and Molybdenum. CRC Press, Boca Raton, FL. Advances in Isotope Methods for the Analysis of Trace Elements in Man. 2001. p. 151-166.

03. Finley, J.W., Ip, C., Lisk, D.J., Davis, C.D., Hintze, K.J., Whanger, P.D. Cancer-protective properties of high-selenium broccoli. Journal of Agricultural and Food Chemistry. 2001. v. 49. p. 2679-2683.

ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0400377 Year: 01 Project Number: 5450-51000-020-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Publications: (Continued)

04. Finley, J.W., Davis, C.D., Hintze, K.J. High-selenium wheat protects against colon cancer in rats. Federation of American Societies for Experimental Biology Journal. 2001. v. 15. p. A62.

05. Hintze, K., Finley, J. Aurothioglucose inhibition of thioredoxin reductase and glutathione peroxidase. Federation of American Societies for Experimental Biology Journal. 2001. v. 15 p. A968.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401877 Year: 01 Project Number: 5450-51000-020-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: SELENIUM IN CONTENT OF BEEF AND WHEAT

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? Y

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

2. How serious is the problem? Why does it matter?

3. How does it relate to the National Program(s) and National Program Component(s)?

4. What were the most significant accomplishments this past year?

This report serves to document research conducted under a trust agreement between ARS and the North Dakota Beef Commission. Additional details of research can be found in the report of the parent project 5450-510000-020-00D.

This project was established to determine if selenium accumulated in the edible beef of cattle raised in high-selenium areas of North Dakota. We found and reported that the concentration of selenium in beef from North Dakota varied over a wide range, depending on the geographical location of the farm or ranch that produced the beef. The concentration of selenium in beef was primarily related to the concentration of selenium in forage consumed by the animal, and there was less of a relationship between selenium in beef and selenium in the soil. The amount of selenium that accumulated in beef was sufficient to allow high-selenium beef to be used as a source of supplemental selenium.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

6. What do you expect to accomplish, year by year, over the next 3 years?

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401877 Year: 01 Project Number: 5450-51000-020-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

9. Scientific Publications:
01. Finley, J.W. Does selenium accumulation in meat confer a health benefit to the consumer? Available from: http://www.asas.org/jas.symposia/proceedings/0911.pdf [2000]

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400398 Year: 01 Project Number: 5450-51000-021-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: BIOAVAILABILITY OF TRACE ELEMENTS, ESPECIALLY IRON FROM FOOD, & ITS INFLUENCE ON NUTRITURE & FUNCTION

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? Y

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Concerns about the adverse effects of iron deficiency on cognition and physical performance as well as concerns about high iron stores and the risk of chronic diseases (such as cancer and heart disease) emphasize the need to determine how dietary iron bioavailability should alter dietary advice for the public.

Both short-term iron absorption and longer-term iron status are being measured in humans consuming controlled diets for several weeks to help determine the true importance of dietary iron bioavailability, and the related impact on practical dietary choices such as consuming less meat, more beans and whole grains, or more tea. The bioavailability of other mineral nutrients, such as calcium, copper, and zinc, may also be affected by such dietary choices, and information on bioavailability of these other mineral nutrients can often be efficiently derived from the same human studies. The iron research will determine the practical importance of dietary iron bioavailability, and how extensively biological adaptation modifies it.

2. How serious is the problem? Why does it matter?

Iron nutrition is a double-edged sword. While adequate iron nutriture in infants, children, and childbearing age women is essential for cognitive development and work capacity, excessive body iron in men and postmenopausal women may increase oxidative stress and the risk of chronic diseases such as heart disease and cancer. Research on dietary iron bioavailability provides information for policy decisions about recommended dietary allowances, food enrichment and fortification standards, and dietary guidelines for the public.

3. How does it relate to the National Program(s) and National Program Component(s)?

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Report of Progress (AD-421)

Accession: 0400398 Year: 01 Project Number: 5450-51000-021-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

National Program 107, Human Nutrition Requirements, Food Composition, and Intake (100%).

This research is directly related to Human Nutrition Performance Goal 3.1.1 Human Nutrition Requirements and 3.1.3 Nutritious Plant and Animal Products, concerning the priority objective: Bioavailability of Nutrients and Food Components.

4. What were the most significant accomplishments this past year?

Concerns about iron deficiency or excess may be moderated if people are able to adapt their iron absorption to differences in dietary iron bioavailability. To further investigate our previous findings that adult men adapt to dietary iron bioavailability, we determined whether women of childbearing age (a group at greater risk of iron deficiency) adapt to variations in dietary iron bioavailability, and whether such adaptation was limited to the specific dietary enhancers and inhibitors in the chronic diet, or constituted a more general change in absorptive efficiency. Premenopausal women absorbed 4-5 times more iron from a high bioavailability diet, compared with a low bioavailability diet, and, in contrast with men, their adaptation to dietary iron bioavailability was minimal, although it was consistent with a general change in absorptive efficiency rather than specific to dietary enhancers and inhibitors. These findings demonstrate the benefit of diets containing lean meat and foods rich in vitamin C, without excessive amounts of phytic acid from legumes, whole grains, and tea, in allowing menstruating women to meet their high iron requirements.

For additional accomplishment related to calcium retention on high meat diets, see report for CRIS project 5450-51530-005-00D.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Determined that people adapted to differences in dietary iron content and bioavailability by adjusting absorption of nonheme iron, the major form of iron in foods, without adjusting absorption of heme iron, the well-absorbed form accounting for 40% of the iron in meat, poultry and fish.

Determined that daily iron supplementation reduces the efficiency of nonheme, but not heme iron absorption from food by about 36%. Found that people with adequate iron stores do not fully adapt to prevent increased iron stores with supplementation, but that women with low iron stores may need

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National Program(s): 107 100%

continuing iron supplementation to counterbalance high rates of iron excretion. Demonstrated that US men partially adapt to differences in dietary iron bioavailability, decreasing their iron retention to less than 1 mg iron per day, despite the high iron bioavailability of their customary diet. Found that adaptation in iron absorption occurred without a change in serum ferritin, the best noninvasive indicator of body iron stores in humans. Demonstrated that research with short-term diets overestimates differences in iron bioavailability between chronic diets. These new findings about adaptation in iron absorption can be helpful in setting recommended dietary allowances for iron.

Determined that women of child-bearing age absorbed substantially less (about 70% less) nonheme iron from a lacto-ovo-vegetarian, compared with an omnivorous diet. Documented that common measures of iron status were unaffected by consuming such a diet for eight weeks, but that fecal ferritin excretion was sensitively affected by dietary iron bioavailability. These findings emphasize the limited response of serum ferritin (a proposed risk factor for cardiovascular diseases and cancer) to dietary iron bioavailability, as well as providing a basis for dietary advice to vegetarians.

Demonstrated that copper is absorbed less efficiently from a vegetarian diet, but because it is especially rich in copper, more total copper is absorbed from a vegetarian than from an omnivorous diet. Together with previous research, these results suggest that vegetarian diets may not be effective in treating copper accumulation disorders.

Demonstrated that fecal ferritin increased with iron bioavailability or iron supplementation in 3 human experiments. This noninvasive gastrointestinal marker can help us understand the intestinal control of iron absorption, and differences in the control of heme and nonheme iron absorption.

Demonstrated that copper, iron and zinc interact to influence body mineral status, that high dietary iron does not induce overt oxidative stress, and that indices of antioxidant capacity are primarily influenced by dietary copper in rats with adequate copper providing the most protection. The results suggest diets high in iron, within a range that could be expected for human diets with iron enrichment, fortification or supplementation, have minimal or no effect on biological oxidation risk factors for chronic disease.

Developed a new method to measure, in humans, the initial intestinal uptake and subsequent transfer to the body of nonheme iron. The results, indicating that initial iron uptake was the major site of absorptive control for nonheme iron,

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National Program(s): 107 100%

impact the development of nutritional guidelines to the public and food industry to help prevent iron deficiency as well as iron overload.

In a cooperative study, the zinc bioavailability of rice provided from the Philippine Rice Research Institute was determined using rats. The findings support the use of brown rice, which would contribute more, although somewhat less bioavailable zinc than the refined forms of rice.

6. What do you expect to accomplish, year by year, over the next 3 years?

This project was discontinued on 3/28/01. Please see the report for project 5450-51000-028-00D for expectations of future accomplishments in this area.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Janet R. Hunt participated as a member of the NAS Institute of Medicine's Standing Subcommittee on Interpretation and Use of Dietary Reference Intakes, beginning July 1, 2000.

USDA research in this area resulted in citation of 15 research articles by JR Hunt (formerly Mahalko) in "Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc" a report from the NAS Institute of Medicine's Food and Nutrition Board released in 2001.

Papers on mineral bioavailability by JR Hunt were cited 12 times in the NIH bibliographic summary, Current Bibliographies in Medicine 99-4: Bioavailability of Nutrients and Other Active Components of Dietary Supplements (http://www.nlm.nih.gov/pubs/cbm/bioavailability.html#400).

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

JR Hunt gave a presentation on "Dietary iron and zinc bioavailability and status: effects of beef" National Cattlemen's Beef Association, Chicago, IL, September 27, 2000.

JR Hunt gave a presentation on "Moving toward a plant-based diet is mineral nutrition at risk?" Annual Meeting of the American Dietetic Association, Denver, CO, October 16, 2000.

ZK Roughead wrote an article, "Getting to the Meat of the

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National Program(s): 107 100%

Matter!" for the Grand Forks Herald, November 8, 2000.
JR Hunt wrote an article, "Winter's Here Are You Getting Your Vitamin D?" for the Grand Forks Herald, December, 2000.

9. Scientific Publications:

01. Hunt, J.R. How important is dietary iron bioavailability? American Journal of Clinical Nutrition. 2001. v. 73. p. 3-4.

02. Hunt, J.R., Dwyer, J. Position of the American Dietetic Association: Food fortification and dietary supplements. Journal of the American Dietetic Association. 2001. v. 101. p 115-125.

03. Hunt, J.R., Roughead, Z.K. Adaptation of iron absorption in premenopausal women consuming diets with high or low iron bioavailability. Federation of American Societies for Experimental Biology Journal. 2001. v. 15. p. A974.

04. Hunt, J.R., Roughead, Z.K. Iron absorption from whole diets: Relationship to iron status, bioavailability, and adaptation in premenopausal women. Proceedings of "Forging Effective Strategies to Combat Iron Deficiency." 2001. Abstract #28. p. 20.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401160 Year: 01 Project Number: 5450-51000-021-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: ADAPTATION IN THE ABSORPTION OF IRON FROM BEEF

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? Y

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Concerns about high iron stores and the risk of chronic diseases such as heart disease and cancer have led to speculation that red meat intake should be limited because it is an excellent source of highly absorbable iron in the heme form. There is special concern that heme iron absorption is not biologically controlled to the same degree as nonheme iron absorption. This project is related to parent CRIS 5450-51000-021-00D by the same investigators, and is limited to a single human experiment that has been partially funded by the National Cattlemen's Beef Association. This experiment determined a) whether heme iron absorption from a meat-based meal is reduced after iron supplementation, b) whether nonheme iron absorption from a meat-based meal is reduced after iron supplementation, c) whether intestinal ferritin production, as measured in fecal samples, is increased after iron supplementation, and is associated with changes in heme and nonheme iron absorption, and d) whether serum ferritin is increased substantially after iron supplementation, and if any increase in serum ferritin persists after iron supplementation is discontinued.

2. How serious is the problem? Why does it matter?

Iron nutrition is a double-edged sword. While adequate iron nutriture in infants, children, and childbearing age women is essential for cognitive development and work capacity, excessive body iron in men and postmenopausal women is hypothesized to increase oxidative stress and the risk of chronic diseases such as heart disease and cancer. Research on dietary iron bioavailability provides information for policy decisions about recommended dietary allowances, nutrient supplementation, food enrichment and fortification standards, and dietary guidelines for the public. Results from this research may suggest that red meat is a good source of iron

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Accession: 0401160 Year: 01 Project Number: 5450-51000-021-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

for persons with inadequate iron nutriture, without providing excessive iron for those with adequate iron stores.

3. How does it relate to the National Program(s) and National Program Component(s)?

National Program 107, Human Nutrition Requirements, Food Composition, and Intake (100%).
This research is directly related to ARS National Human Nutrition Performance Goals 3.1.1, Human Nutrition Requirements, and 3.1.3, Nutritious Plant and Animal Products, concerning the primary objective: Bioavailability of Nutrients and Food.

4. What were the most significant accomplishments this past year?

In the past year, the only activity for this project has been the final scientific publication of the project's results (see publications, below).

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Iron intakes in the US have increased because of increased fortification of foods and use of nutrient supplements. To help understand how well the body adapts to changes in iron intake, we investigated how well men and women adapted to supplementation with 50 mg iron daily for 12 weeks. Healthy people, even those with low iron stores, reduced nonheme, but not heme, iron absorption from food in response to iron supplementation. Fecal ferritin increased with iron supplementation, but this was apparently unrelated to heme iron absorption. Yet those with adequate iron stores did not fully adapt to prevent increased iron stores with supplementation, and those with low iron stores rapidly reverted to their previous iron status after supplementation was discontinued. Heme iron may be an important source of bioavailable iron for women with low iron stores. This research contributes to a better understanding of control of body iron retention and emphasizes the need for dietary guidelines and fortification or supplementation practices that protect against iron deficiency as well as iron excess.

6. What do you expect to accomplish, year by year, over the next 3 years?

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Accession: 0401160 Year: 01 Project Number: 5450-51000-021-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

9. Scientific Publications:

01. Roughead, Z.K., Hunt, J.R. Adaptation in iron absorption: iron supplementation reduces nonheme, but not heme iron absorption from food. American Journal of Clinical Nutrition. 2000. v. 72. p. 982-989.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400524 Year: 01 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Title: HUMAN MINERAL ELEMENT REQUIREMENTS AND THEIR MODIFICATION BY STRESSORS

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? Y

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The extent to which dietary mineral excesses, deficiencies and imbalances contribute to the susceptibility and severity of a number of chronic diseases of major health and economic consequence has not been established; the chronic diseases include coronary heart disease, hypertension, stroke, atherosclerosis, and osteoporosis. Additionally, the extent to which mineral element nutrition contributes to these diseases is unknown when nutritional, metabolic, hormonal or physiological stressors are present which could enhance the need, or interfere with the utilization of the mineral elements. There is a need to ascertain the validity of claims that magnesium is of practical concern for maintaining bone and cardiovascular health; that boron, copper, zinc and manganese status affects calcium utilization and metabolism and thus the susceptibility of osteoporosis; and that low and/or high dietary zinc adversely affect copper metabolism resulting in an increased risk to cardiovascular disease.

Studies with human volunteers are being and will be conducted. These studies include examining the effects of varying intakes of zinc at different intakes of dietary copper on lipid profiles, bone status indicators and reactive oxygen metabolism; whether low magnesium intakes with and without stressors results in a neurogenic inflammatory response leading to oxidative damage that can lead to pathophysiology such as cardiomyopathy, migraine headaches, and abnormal central nervous system function; and whether boron supplementation of individuals with suspected low boron status improves cognitive and motor function and indicators of bone health.

2. How serious is the problem? Why does it matter?

Dietary factors, including trace element nutriture, are associated with 5 of the 10 leading causes of death. Among the

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Accession: 0400524 Year: 01 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

diseases that are closely linked to diet, the cost for treatment and care in the United States exceeds $200 billion per year. Among the diseases associated with subnormal mineral element nutrition, the annual economic cost is estimated to be greater than $80 billion for cardiovascular disease, and $10 billion for osteoporosis. Several mineral elements associated with these chronic diseases including copper, magnesium and zinc have been shown to be routinely low in the diets in the United States. Thus, providing information about requirements and factors that affect those requirements of critical mineral elements should result in policies and programs that improve intakes of these nutrients and thus result in a healthier population, a decrease in the burden of chronic disease, an enhancement in the quality of life, and a diminishment in health care expenditures.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research relates to the National Program 107, Human Nutrition, and specifically to the program component of Nutrient Requirements. The research helps determine mineral requirements that prevent disease and promote health and optimal function throughout life. The major focus is on determining the biochemical and health consequences of suboptimal mineral intakes, with the objective of showing that mineral nutrition influences the major chronic, degenerative conditions associated with aging.

4. What were the most significant accomplishments this past year?

A. In the past, controlled experiments with human subjects conducted to show that a dietary magnesium deprivation results in changes in biochemical and physiological indices associated with some of the well known enzyme functions of magnesium which could lead to pathological consequences have not been particularly successful. Thus, human volunteers were fed diets containing inadequate, marginal and adequate amounts of magnesium over a six month period to ascertain whether a systemic neurogenic inflammatory response is of primary importance during magnesium deprivation. Magnesium deprivation resulted in elevated circulating substance P; and changes in the release of other neurogenic peptides, including decreases in calcitonin gene-related peptide and neuropeptide Y; and an apparent increase in oxidative stress as indicated by reduced circulating glutathione and increased extracellular superoxide dismutase. These changes may be the reason that

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400524 Year: 01 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

numerous epidemiological findings and magnesium supplementation trials show that a low magnesium status is associated with numerous disorders including coronary heart disease, hypertension, migraine headaches, sleep disorders, mood disturbances, and osteoporosis.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Low zinc, low copper, and especially the combined low zinc and copper caused unfavorable changes in bone and calcium status indicators including urinary N-telopepetides and calcium, and plasma osteocalcin and ionized calcium. The findings indicate that increasing the dietary intake of copper and zinc is an action a significant number of people could do to reduce the risk of developing osteoporosis.

Consumption of a diet rich in polyunsaturated fat (vegetable fats tend to be more polyunsaturated) significantly increased plasma ionized calcium, ionized magnesium and total magnesium concentrations and significantly decreased plasma iron concentration and transferrin saturation in postmenopausal women. The findings show that changes in dietary fat can modify the metabolism of minerals associated with bone maintenance and growth and with iron utilization. Routine consumption of a diet high in polyunsaturated fats may help reduce the risk of osteoporosis, but also may increase the risk of becoming iron deficient if iron intake is low.

Experimental evidence using human volunteers shows that low dietary intakes of magnesium induces changes in indices used to assess the susceptibility to cardiovascular and calcium metabolism disorders; changes induced include heart rhythm abnormalities, altered cardiovascular function and energy metabolism in postmenopausal women. Because magnesium is often consumed in inadequate amounts according to dietary surveys, these findings indicate that magnesium is of practical nutritional and clinical importance in the maintenance of healthy cardiovascular system, and thus in the prevention of heart disease.

Calcium balance can be maintained in postmenopausal women with intakes less than 800 mg/day, but can be undesirably altered by low intakes of copper, zinc and magnesium. These findings show that high dietary intakes of calcium are not the complete answer to the prevention of bone loss leading to osteoporosis. If nutrients such as magnesium, copper, zinc and boron are consumed in appropriate amounts, high dietary calcium intakes (such as those indicated by the new Dietary

ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0400524 Year: 01 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Reference Intakes) difficult to achieve by diet alone may be unnecessary to prevent bone loss in postmenopausal women.

The consumption of high amounts of fructose decreases calcium balance with the effect more marked when dietary magnesium is low. This finding indicates that the consumption of high amounts of carbonated beverages sweetened with high fructose corn syrup is detrimental to the formation and maintenance of healthy strong bones.

High dietary zinc (53 mg/day) compared to low dietary zinc (3 mg/day) significantly decreased plasma cholesterol and significantly increased a number of indicators of copper status including platelet cytochrome c oxidase activity and ceruloplasmin concentrations in postmenopausal women fed low (1 mg/day) or luxuriant (3 mg/day) copper. These findings do not support the dogma that a moderately high dietary zinc intake adversely affects copper metabolism, but show that a low dietary zinc intake does. Combined low intakes of copper and zinc may be important factors in the occurrence of heart disease.

6. What do you expect to accomplish, year by year, over the next 3 years?

This project was discontinued. Please see the report for project 5450-51000-030-00D for expectations of future accomplishments in this area.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Information about the nutritional and clinical importance of the mineral elements magnesium, copper, boron and zinc as it becomes available is routinely transferred to a variety of customers. The customers include the public through web pages of professional organizations and via the popular media, and other scientists through presentations at national and international meetings and professional publications.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Research findings pertaining to magnesium findings cited in the popular media and periodicals such as Bottom Line Personal. No attempt is made to keep a record of these

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400524 Year: 01 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

articles.

9. Scientific Publications:
01. Nielsen, F.H. Other trace elements. Kiple, K.F., Ornelas, K.F., editors. Cambridge University Press, Madrid, Spain. The Cambridge World History of Food. Volume 1. 2000. p. 856-868.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149894 Year: 01 Project Number: 5450-51530-004-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 25% 5.3.1.2 75%
National Program(s): 107 100%

Title: DEVELOPMENT AND EVALUATION OF METHODS FOR THE CLINICAL EVALUATION OF MINERAL NUTRITIONAL STATUS

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? Y

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Although the essentiality of magnesium, copper and zinc is well established, the general consensus in the mineral research community is that there are currently no adequate clinical tests for evaluating the nutritional status of these elements in humans. Moreover, the lack of accurate diagnostic tests to assess magnesium, copper and zinc status has impeded the development of robust well-founded dietary guidance for these essential elements, and impeded the identification of chronic diseases such as ischemic heart disease and osteoporosis, whose incidence and severity may involve imbalanced or deficient intakes of these essential mineral elements.

The approach to resolving the problem is to feed human volunteers deficient or imbalanced intakes of copper, zinc and/or magnesium, measure variables that can be ethically assessed and are responsive to deficient or imbalanced intakes based on findings from animal models, and compare the values obtained to those obtained when intakes of these elements are balanced and adequate.

2. How serious is the problem? Why does it matter?

Food consumption surveys indicate that a significant number of people in the United States have intakes of copper, zinc and/or magnesium that are below those recommended by the Food and Nutrition Board of the National Academy of Sciences. Yet, pathology caused by deficiencies of these elements is not readily recognized or diagnosed because of the lack of definitive methods for identifying deficient states. Establishment of specific, accurate and cost-effective tests for the measurement of nutritional status will aid in detecting nutritional deficiencies and imbalances in their early stages. Early detection of inadequate trace element status will result in considerable savings in the cost for

ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0149894 Year: 01 Project Number: 5450-51530-004-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 25% 5.3.1.2 75%
National Program(s): 107 100%

treatment and care of diet-related diseases in the United States that has been estimated to exceed $200 billion per year. Additionally, methods to assess status would be applicable to studies determining human requirements and metabolism of trace elements. Knowledge gained from this research project will facilitate the evaluation of federal food and nutrition programs, and the administration of programs that contribute to the health and well-being of people.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research relates to the National Program 107, Human Nutrition, and specifically to the program component of Nutrient Requirements which states research is needed to identify biomarkers of nutrient intakes and status, nutritional adequacy and disease prediction. The research emphasizes priority objectives of Biomarkers, and Function and Performance, but also can apply to objectives of Mechanism of Action and Nutrient Interactions.

4. What were the most significant accomplishments this past year?

The SY associated with this project retired.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Plasma zinc, extracellular superoxide dismutase, erythrocyte membrane 5'-nucleotidase, and bone specific alkaline phosphatase were shown to be sensitive indicators of zinc nutritional status in postmenopausal women fed 3 then 53 mg of zinc for 90 days each. These indicators should be helpful in defining human zinc requirements during different stages of the life cycle, and for surveys evaluating zinc status of populations at risk to chronic disease in which suboptimal zinc intake plays a role.

Magnesium status indicators were determined in a double blind crossover study in which postmenopausal women were fed low and deficient magnesium and low or deficient copper. The sequence in which magnesium supplements were given obscured the magnesium effects on variables such as serum magnesium, serum ionized magnesium, serum cholesterol, plasma glucose, and red cell superoxide dismutase; changes were greatest when the placebo was fed first and were smaller or lacking when

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Accession: 0149894 Year: 01 Project Number: 5450-51530-004-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 25% 5.3.1.2 75%
National Program(s): 107 100%

magnesium was fed first. The findings suggest that prior high magnesium intake and status inhibits or delays the appearance of magnesium deprivation signs. This information is useful in the development of further studies with the objective of determining magnesium nutritional requirements, and defining the consequences of magnesium deprivation.

High dietary fructose significantly increased magnesium balance and the urinary loss of phosphorus and depressed both calcium and phosphorus balances, with the effects more marked when dietary magnesium was low. These findings suggest that high dietary fructose adversely affects calcium and phosphorus metabolism in humans, particularly when dietary magnesium is low. This is of concern because recent surveys indicate that the consumption of fructose-based sweeteners is high and that a significant portion of the population is consuming diets low in magnesium. The findings suggest that high fructose intakes, such as those with high intakes of soda pop and sport drinks, coupled with a low dietary magnesium intake could result in the loss of bone, or osteoporosis.

6. What do you expect to accomplish, year by year, over the next 3 years?

The project should be terminated; it is bridged to 5450-51000-007-00D.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

New information on nutritional and clinical importance of status indicators for zinc, copper and magnesium is routinely transferred to a variety of customers.

These customers include the public through web pages of professional organizations and the Grand Forks Human Nutrition Research Center, and via the popular media, and other scientists through presentations at national and international meetings and professional publications.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Tracking of research findings cited in periodicals is not routinely done, but it was observed that the finding that high fructose intakes, such as that with a high consumption of soda

ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0149894 Year: 01 Project Number: 5450-51530-004-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 25% 5.3.1.2 75%
National Program(s): 107 100%

pop and sport drinks, adversely affects calcium metabolism and thus bone was cited in a newsletter, Bottom Line Personal.

9. Scientific Publications:

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

UNOFFICIAL

MINERAL NUTRIENT FUNCTIONS

MANAGEMENT UNIT

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400186 Year: 01 Project Number: 5450-51000-018-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.1.3.4 25%
National Program(s): 107 100%

Title: DIETARY TRACE ELEMENTS AND PHYSIOLOGY OF THE CARDIOVASCULAR AND RELATED SYSTEMS

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? Y

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Dietary copper deficiency causes biochemical deficits and structural damage to the cardiovascular system (heart and circulation). Although progress is being made in identifying defects in cardiovascular physiology (function) in copper deficiency, the mechanisms for these functional changes are not completely clear. The known functional changes are not related in a clear fashion to the known structural and biochemical changes. Additionally, because most of the studies on dietary copper have not been done in humans or with intakes consistent with human consumption, the relationship of such findings to human health is not clear. The approaches to resolving this problem include:

A. Determination of functional changes in blood vessels and, in particular, clarification of changes in signal transduction pathways in smooth muscle and endothelium caused by dietary copper deficiency. Relevant studies will be performed with isolated vessels, on isolated organs and on whole animals. The ultimate goal is to determine the contribution of adequate copper nutrition to maintenance of blood flow to organs and to maintenance of blood pressure.

B. Identification of functional changes in the heart and their relationship to metabolic and biochemical alterations caused by trace element (copper) deficiencies. The focus will be to determine coronary blood vessel and cardiac muscle vulnerability to physiologic and metabolic stressors including, but not limited to, adrenaline stimulation and simulated heart attack (cessation and re-starting of blood flow to the heart). Isolated heart and whole animal models will be used.

C. Elucidation of general biochemical mechanisms of damage caused by copper deficiency. Oxidative stress continues to be a strong, although somewhat equivocal, candidate as a mechanism for generalized damage. Damage by oxidative mechanisms will be compared with that caused by another mechanism, glycation. The aim is to attempt to relate known enzymatic, metabolic and hormonal changes to the deterioration of function that occurs in copper deficiency. Various organs will be tested, but the primary focus will be on the heart and blood.

2. How serious is the problem? Why does it matter?

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Report of Progress (AD-421)

Accession: 0400186 Year: 01 Project Number: 5450-51000-018-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.1.3.4 25%
National Program(s): 107 100%

Studies to date have indicated that dietary copper deficiency has considerable potential for contributing to chronic disease (for example, ischemic heart disease, atherosclerosis, high blood pressure) and the debilitating effects of aging. Experimental evidence indicates that a third or more of the American population may be consuming less than the Estimated Safe and Adequate Dietary Intake of copper set by the Food and Nutrition Board of the National Academy of Sciences. Research will provide information that will be used to set recommendations for dietary copper based on a reduction in risk of chronic disease, particularly of the heart and blood vessels.

3. How does it relate to the National Program(s) and National Program Component(s)?

These studies directly contribute to National Program 107 (100%). Relative to the new Human Nutrition Action Plan, these studies will address elements of Performance Goal 3.1.1 Human Nutrition Requirements, with specific emphasis on identifying potentially beneficial mechanisms of action of trace elements and characterizing the role of trace elements in achieving optimal physiologic function.

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishment during FY 2001:
A review was published that 1) consolidated the mechanistic findings of the laboratory on the cardiovascular effects of dietary copper deficiency and 2) provided a focused hypothesis and direction for future studies. The integration of three mechanisms, peroxidation, glycation, and nitration, that were previously examined independently, has illustrated the complexity of the defects caused by copper deficiency and further indicates the dependence of good cardiovascular health upon adequate dietary copper.

B. Other Significant Accomplishment(s):
Dietary copper deficiency causes impaired immune function, but the defect has not been completely characterized. A necessary component of the immune response is the movement of immune cells called leukocytes from the blood to the site of injury or infection. To do so they must bind to the blood vessel wall and move into the affected tissue. We have found that the adhesion of leukocytes to the blood vessel wall is reduced in copper-deficient rats, which would be expected to impair their immune response. This finding adds to the known mechanisms by which copper deficiency is known to affect immune function and contributes to our knowledge of the importance of adequate copper nutrition. (Collaborator - D.A. Schuschke, University of Louisville).

C. Significant Accomplishments/Activities that Support Special Target Populations.

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Accession: 0400186 Year: 01 Project Number: 5450-51000-018-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.1.3.4 25%
National Program(s): 107 100%

None.

D. Progress Report.
None.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

We have shown that the concentrations of two important chemical messengers, nitric oxide and cyclic GMP, are elevated in copper-deficient hearts, which suggests their possible role in reduction of heart contractile force in copper deficiency. A subsequent study showed that the amount of one of the enzymes that produces nitric oxide, inducible nitric oxide synthase, is also elevated in copper deficiency, which suggests an elevated genetic expression of this enzyme. These studies help to define the molecular basis for impaired heart function when dietary copper is restricted.

Strong evidence was found supporting the view that glycation, the undesirable binding of sugar to proteins, is enhanced in dietary copper deficiency. Blood analysis revealed the presence of glycated hemoglobin and fructosamine (blood proteins with sugar bound to them) as well as pentosidine (a product of blood protein damaged by glycation). Because glycation is a process that is increased in diabetes and aging, this finding suggests that reduced copper intake may worsen the consequences of these two conditions.

Measurements of heart and blood vessel function in copper deficient animals helped to show that, although cardiac output was not altered by copper deficiency, blood vessel resistance was reduced and volume of blood ejected per beat (stroke volume) of the heart was elevated; the higher stroke volume may contribute to the pathologically greater size of copper-deficient hearts. These and succeeding physiological measurements will help to characterize heart function in dietary copper deficiency.

Studies with collaborators on the effect of copper deficiency on blood clotting function have shown that the aggregation of blood platelets to one another was increased and that adhesion of platelets to blood vessel endothelial cells was reduced. Further, these findings were associated with an alteration of two platelet clotting factors, fibrinogen and von Willebrand factor. These studies emphasize the importance of dietary copper to prevent bleeding.

Another collaborative study found that the dilation of blood vessels in response to an inflammatory agent was exaggerated in copper-deficient rats. By use of appropriate blocking agents, the potential mechanism(s) responsible for this change were delineated. This study shows the importance of proper copper intake in mediating the body's inflammatory response to injury.

6. What do you expect to accomplish, year by year, over the next 3 years?

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Report of Progress (AD-421)

Accession: 0400186 Year: 01 Project Number: 5450-51000-018-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.1.3.4 25%
National Program(s): 107 100%

FY 2002: We will examine whether and to what extent the nitric oxide signal transduction pathway is involved in the altered function of copper-deficient hearts; this will include examination of nitric oxide effects on contractile function and mitochondrial respiration as well as cardiac genetic expression of the enzymes that produce nitric oxide in copper-deficient hearts. FY 2003: Research will be aimed at examining the relationship between the alteration of blood vessel function and regulation of blood pressure in copper-deficient animals. This will focus on the known alteration of nitric oxide-dependent mechanisms as well as mechanisms indirectly affected by altered nitric oxide metabolism. FY 2004: Research will be directed toward determining whether a direct association can be made between formation of advanced glycation end-products and altered cardiovascular function of copper deficiency; examination of heart and blood vessel function will be made in the presence of known inhibitors of glycation. Because a positive finding in the latter study would suggest altered carbohydrate metabolism, we will examine the role of the pancreas in initiating cardiovascular effects of dietary copper deficiency; this would include examination of the role of nitric oxide on pancreatic function (i.e., hormone release)and whether the resulting alteration of carbohydrate metabolism can be shown, by functional testing, to account for cardiovascular effects.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Nothing to report.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Nothing to report.

9. Scientific Publications:

01. Saari, J.T. Copper deficiency and cardiovascular disease: role of peroxidation, glycation and nitration. Canadian Journal of Physiology and Pharmacology. 2000. v. 78. p. 848-855.

02. Schuschke, D.A., Saari, J.T., Miller, F.N. Leukocyte-endothelial adhesion is impaired in the cremaster muscle microcirculation of the copper-deficient rat. Immunology Letters. 2001. v. 76. p. 139-144.

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Report of Progress (AD-421)

Accession: 0400186 Year: 01 Project Number: 5450-51000-018-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.1.3.4 25%
National Program(s): 107 100%

Publications: (Continued)

03. Schuschke, D.A., Saari, J.T. Dietary copper deficiency impairs nitric oxide-mediated signal transduction. Recent Research Developments in Nutrition. 2000. v. 3. p. 65-70.

04. Saari, J.T., Dahlen, G.M. Dietary copper deficiency causes elevation of early and advanced glycation end-products. Roussel, A.M., Anderson, R.A., Favrier, A.E., editors. Kluwer Academic/Plenum Publishers, New York, NY. Trace Elements in Man and Animals 10. 2000. p. 523-526.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400106 Year: 01 Project Number: 5450-51000-019-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 90% 5.1.4.3 10%
National Program(s): 107 100%

Title: MINERAL ELEMENT NUTRITION, NEUROPSYCHOLOGICAL FUNCTION AND BEHAVIOR

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? Y

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The consequences of mild to moderate mineral deficiencies for neuropsychological function and behavior are largely unknown. This represents a serious problem because suboptimal mineral intakes and status have been linked to chronic disorders such as depression and dementia. Further, national nutrition surveys indicate that dietary intakes of several essential minerals are less than recommended in many segments of the U.S. population and existing data are frequently inadequate or unavailable to make recommendations based on functional outcomes. This project addresses the need for increased experimentally-derived knowledge leading to a better understanding of the relationships among mineral element nutrition, neuropsychological function and behavior. Such knowledge is critical when making recommendations for mineral intakes that will facilitate optimal neuropsychological health and performance throughout the life span in all segments of our population. Behavior is unique as a criterion for establishing nutritional adequacy because it represents the functional integration of all biological systems, including compensatory mechanisms that often determine the practical importance of a nutritional deficit or excess.

Neuropsychological and behavioral consequences of mild and moderate deficiencies in biologically essential mineral elements are determined with the goal of improving health, work and school performance, and sense of well-being in the population. Specifically, studies are designed to determine: the role of mineral elements in cognition (i.e., attention, perception, learning, memory and reasoning) and spatial and motor skills; the effect of mineral nutrition on mood states and emotional and social adjustment; the impact on nutrition-behavior relationships of potential mediating factors, including environmental and endogenous stressors like noise, temperature, sleep duration and quality, and menstrual and menopausal symptoms; and, the effect of mineral nutrition on electro-physiology indexing brain function to gain insights into the mechanisms for nutritional effects on performance and sense of well-being. New methods and technologies are developed to increase efficacy of behavioral assessments and promote their use by other nutrition scientists. Studies of healthy adults and children are complemented by animal studies.

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Accession: 0400106 Year: 01 Project Number: 5450-51000-019-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 90% 5.1.4.3 10%
National Program(s): 107 100%

2. How serious is the problem? Why does it matter?

Findings obtained during the past 40 years indicate that the mineral elements boron, copper, iodine, iron, magnesium, manganese, selenium and zinc likely are important for normal neuropsychological function and behavior of adults and children. However, previous studies have yet to establish the reliability of effects of graded mineral intakes on behavior or to adequately characterize the relationship between mineral element nutrition and brain function and cognition. Such information is critical to characterizing the mechanisms responsible and is needed to apply findings to real-world problems. To respond to intense public interest in the relationship between nutrition and performance, and potentially to improve public health, productivity and sense of well-being, there is a great need to increase our knowledge of the functional consequences of graded intakes of mineral elements, and especially the consequences of marginal intakes common in many segments of the population. Food consumption surveys indicate that intakes of calcium, copper, iron, magnesium and zinc are significantly below the RDA or ESADDI for large segments of the adult population in the United States and worldwide, and many reviews have concluded that mild-to-marginal deficiencies in these and other mineral elements are particularly likely in the groups targeted by this research (e.g., women, children, elderly). Further, increased knowledge of the relationship between mineral element nutrition, neuropsychological function and behavior is needed for a more complete determination of nutrient requirements, establishing recommended dietary intakes, and evaluating the efficacy and adverse effects of taking dietary supplements, a multi-billion dollar industry in the United States.

3. How does it relate to the National Program(s) and National Program Component(s)?

This research relates to program 107, Human Nutrition (100%). The program component most directly addressed by this project is Nutrient Requirements, with objectives including identifying and developing indicators of function and performance that are sensitive to changes in nutrients and bioactive components of the diet, and characterizing the role of nutrients and other dietary components in achieving and maintaining optimal physiologic and psychologic function and performance. This research also addresses the objectives to determine the functional impact of interactions among dietary constituents and among nutrients and lifestyle, environmental and genetic factors. Products of this research also relate to the program components, Relationship between Diet, Genetics and Lifestyle and the Risk for Chronic Disease, and Health Promoting Intervention Strategies for Targeted Populations.

The determination of dietary requirements for optimal cognitive function and performance has been identified as a national need. This research

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Accession: 0400106 Year: 01 Project Number: 5450-51000-019-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 90% 5.1.4.3 10%
National Program(s): 107 100%

furthers ARS objectives by directly evaluating, under highly controlled conditions, the effects of mineral element nutrition on neuropsychological function and behavior of adults, including the elderly, and in children and adolescents. This research facilitates the detection of mild mineral deficiencies and helps define dietary mineral requirements to develop and maintain health and optimal function throughout the life cycle. Examining the combined effects of nutritional insults and exogenous and endogenous stressors offers insights into ways to improve performance in work and school, and in other situations with a high demand. Understanding the true role of mineral element nutrition in neuropsychological function and behavior also helps individuals and groups to more knowledgeably evaluate nutrition claims, and promotes healthy and cost-efficient dietary behavior.

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishment during FY 2001:
Although chromium (Cr) supplementation has increased dramatically in recent years (primarily based on claims that Cr promotes weight loss and increases muscle mass) and it may improve insulin efficiency and lipid profiles in individuals with impaired glucose tolerance, the psychological effects of Cr supplementation have not been systematically investigated. Neuropsychological function (attention, perception, memory, reasoning and psychomotor function) and menstrual symptomatology (physical and psychosocial) were assessed in 83 women (aged 18-50 y) prior to and following 12 weeks double-blind supplementation with either 195 mcg/d Cr picolinate, 1700 mcg/d picolinic acid, or a placebo. In addition to the expected increases in plasma and urinary Cr with the Cr picolinate supplement, compared to placebo, both active supplements increased reported severity of intermenstrual anxiety, depression and negative mood, and premenstrual pain, water retention and negative mood. Findings demonstrate that supplements containing picolinic acid may have negative consequences for menstrual symptomatology and that Cr supplementation in physiologic amounts does not benefit menstrual symptomatology, cognitive function or psychomotor performance; findings thus provide needed information about the functional consequences, including adverse effects, of Cr supplementation useful to scientists, health care providers, policy makers and the public.

B. Other Significant Accomplishments:
None.

C. Significant Accomplishments/Activities that Support Specific Target Populations:
None.

D. Progress report:
Note accomplishment described in 4.A. above. This project was terminated

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400106 Year: 01 Project Number: 5450-51000-019-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 90% 5.1.4.3 10%
National Program(s): 107 100%

04-11-01 and replaced with the bridging project, Mineral Element Nutrition, Neuropsychological Function and Behavior (CRIS No. 5450-51000-027-00D), effective that same date.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Demonstrated a relationship between zinc nutrition and adaptive functioning, behavior problems and school performance of children (aged 6-9 years) living in Brownsville, TX and at risk for Zn deficiency because of life stage and regular consumption of cultural foods high in phytate. Hair Zn concentration was negatively associated with teacher-reported anxiety and depression, withdrawal and total adjustment problems, indicating that problems associated with lower Zn status were personality rather than conduct in nature. Findings provided the first evidence that Zn status is predictive of mood disturbances and behavior problems in school-aged children, and point to the need for further study to determine whether increased Zn intakes might prevent or help alleviate mood and behavior problems that directly affect school performance, cognitive and social development, and quality of life for many children.

Biochemical indices of B12 status were determined in at-risk Guatemalan school children (aged 8-12 years) and statistically related to measures of adaptive functioning, behavior problems and school performance. Methylmalonic acid and homocysteine indicated a positive relationship between B12 status and academic performance and adaptive function, and a negative relationship between B12 status and attention problems, while folate indicated a negative relationship between B12 status and withdrawal, somatic complaints and social problems. These behavioral effects complement findings that children with low and marginal B12 status also performed less well on computerized neuropsychological tests to provide the first evidence that B12 deficiency is associated with cognitive and behavioral impairment in children as well as in the elderly.

Healthy premenopausal women were fed either 1 or 20 mg/day manganese for 8 weeks as part of a highly controlled metabolic unit study and examined double-blind by a licensed neurologist for the occurrence and severity of more than 40 neurologic signs and symptoms. Neither dietary group showed any signs or symptoms. This finding indicates that dietary intakes in the range consumed by many vegetarians (10-17 mg/day) are unlikely to result in neurologic problems and underscores the need to coordinate efforts to establish recommended dietary intakes and to set regulatory guidelines (the current Reference Dose for Mn is 10 mg/day) to protect the population from toxicologic effects of minerals naturally found in the diet.

Demonstrated that low copper (Cu) intake (1 versus 3 mg/day) can impair short-term verbal memory of healthy postmenopausal women, indicated by increased intrusions during verbal recall and finding that Cu status indicators (plasma Cu and ceruloplasmin) were positively correlated with

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and predicted improved verbal memory, improved long-term memory, increased clustering of related verbal material (strategy) and fewer intrusions during recall (reduced distraction). A moderately high zinc (Zn) intake (53 mg/day) combined with a low Cu intake, improved list recall, but impaired verbal span and a general measure of short-term memory. These data increase our understanding of the role of Cu nutriture in cognitive function, which has received little study, and address the interactive effects of moderately high Zn intakes combined with low Cu intakes. The impact of these findings was to spur additional study of the relationship between dietary Cu and cognitive function, including dementia, with the potential impact to affect recommended intakes and improve cognitive function in the elderly and other populations.

Determined that short-term supplementation with zinc (Zn) combined with other micronutrients may improve some aspects of cognitive function of school-aged Mexican-American children, who are at increased risk for Zn deficiency primarily because of high intakes of dietary phytate. Children (aged 6-9 years) living in Brownsville, TX were treated with a micronutrient (M) mixture containing 50% RDA or mean ESSADI vitamins and minerals (excluding iron, calcium, magnesium, phosphorus and Zn, with folate at 25% RDA), 20 mg/d Zn plus micronutrients (Zn+M), 20 mg/d iron plus micronutrients, or a placebo (P) for 10 weeks in a double-blind control trial. Compared to the other treatments and P, Zn+M improved reasoning, indicated by fewer number of trials needed to learn simple concepts. This research complements earlier work by this laboratory that found a similar treatment regimen improved cognitive and psychomotor function of Chinese children with a high incidence of Zn deficiency primarily caused by inadequate Zn intakes. Findings provide further indication that Zn supplementation may significantly benefit cognitive function in deficient populations, and eventually may be used in setting recommended intakes for Zn and formulating school meal programs. While it is known that Zn is essential for growth and early development, these data provide evidence that Zn intake is important to cognitive function in later development.

Determined that zinc (Zn) deprivation (4.5 mg/day for 2 months) affected short-term verbal memory of healthy adult men. Reaction times to recognize previously learned words were initially increased and then decreased in response to deprivation, which suggests adaptation to reduced Zn intakes. Numerous other aspects of cognitive function and psychomotor performance were also evaluated, but no evidence was found for any other effects of low dietary Zn intakes in this sample. Findings complement previous research showing that Zn nutriture may affect cognitive function in the adult, provide experimental evidence that homeostatic controls may compensate for short-term cognitive effects during chronic Zn undernutrition, and indicate that memory is the cognitive process most sensitive to Zn deprivation. Findings help other scientists focus the goals and approach of future studies of Zn and cognitive function.

Determined that zinc (Zn) and micronutrient supplements improved

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National Program(s): 107 100%

cognitive and psychomotor function of rural and urban Chinese school children. Motor skills, including manual dexterity and eye-hand coordination, visual perception, memory for simple shapes and complex objects, and reasoning were functions most affected by Zn (20 mg/day) and Zn combined with other micronutrients at 50% recommended intakes. Findings indicate that cognitive and psychomotor function, and thus school performance, may be suboptimal in the >25% of Chinese children and 6-10% of school-aged children in the United States who are Zn deficient. This was the first evidence that Zn supplementation of young children may improve cognition and psychomotor function. Findings were widely reported by the national media, followed by many inquiries from the public, private industry and granting agencies, and by invitations to speak on this research. This research led directly to collaborative studies of cognitive and psychomotor effects of Zn supplementation of Mexican-American school children (supported by grants from the Gerber Foundation and USDA National Research Initiative), of zinc and iron supplementation of young women (supported by grant from the US Army Medical Research, Development, Acquisitions and Logistics Command), and of Zn deprivation of young adult men (supported by USDA ARS Western Human Nutrition Research Center).

Experimentally determined that adult male rats fed diets deficient (1 microgram/g diet) or excessive (100 mcg/g diet) in manganese (Mn) were generally less active than those fed adequate Mn (10 mcg/g diet), and consequently, these rats engaged in fewer aggressive behaviors (attacking, biting, wrestling, aggressive contact) and displacement activities (exploration, self-grooming), and more posturing. However, rats fed diets high in Mn but low in calcium (Ca; 2500 versus 5000 microgram/g diet) did show increased aggressive behavior compared to rats fed other diets. Findings provide weak support for earlier reports that Mn excess increases aggression, primarily because rats fed high Mn were less active than those fed adequate Mn. No support was found for the hypothesis that Mn deprivation is associated with increased aggression. Mn is essential for normal brain function and behavior, and there has been speculation that moderate Mn intoxication or deficiency may be associated with increases in aggressive behavior. Findings from this study expand knowledge of the functional role of dietary Mn, and its interaction with Ca, at physiological as well as toxicological concentrations. Predicted impact is redirection of future research on possible nutritional involvement in aggressive behavior to study the interaction of mineral nutrients.

Showed that dietary selenium (Se) affects mood states of healthy adults. Men in the United States with typically adequate Se intakes and fed approximately 3 times the RDA for Se for 12 weeks reported less depression and mental confusion than men fed approximately one-third the RDA. Women in New Zealand with typically low Se intakes and supplemented daily with 40 ug Se reported more energy and confidence, less hostility, and a decrease in total mood disturbance after 15 weeks. Findings indicate a novel function for dietary Se that may be used to help establish Se requirements for adults. Findings were widely reported by the national media, followed by

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numerous inquiries from other researchers and the general public. Extension of this research to assess cognitive performance and brain electrical activity in response to changes in Se intakes is a component of a recently awarded grant from the USDA CSREES National Research Initiative Program to study the efficacy of feeding Se-enhanced meat, wheat and broccoli to healthy adults in the Peoples Republic of China.

Determined in mature rats that low dietary intake for 10 weeks of either copper (Cu)(0.05 versus 6.0 mcg/g)and magnesium (Mg)(50 versus 500 mcg/g)was associated with an increase in generalized activity. Low Cu intake also resulted in more stereotypic behavior during presentation of an auditory stressor, whereas low Mg intake resulted in increased stereotypic behavior regardless of the presence of the stressor. Low Cu intake was associated with poorer performance on measures of learning, whereas low Mg intake was associated with poorer performance on measures of memory. Neither Cu nor Mg showed strong effects on direct measures of anxiety; however, indirect measures of stressor effects during activity monitoring and memory testing suggest that both minerals may impact emotionality. Cu and Mg are two minerals of potential relevance to behavior because of their importance in neurotransmitter metabolism and because previous studies have shown that dietary intakes of both minerals affect brain electrophysiology. Findings indicate that both Cu and Mg have functional consequences at the behavioral (and possibly emotional) level, which complement earlier findings of effects of these two minerals on brain physiology. Predicted impact is increased efficacy of future research on neuropsychological function and behavior of humans.

6. What do you expect to accomplish, year by year, over the next 3 years?

Expected accomplishments during FY 2002-2004: This project was terminated 04-11-01 and replaced with the bridging project, Mineral Element Nutrition, Neuropsychological Function and Behavior (CRIS No. 5450-51000-027-00D), effective that same date.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

As member of the DRI Panel on Micronutrients (Food and Nutrition Board, Institute of Medicine, National Academy of Sciences), reviewed and interpreted scientific literature and expert testimony on arsenic, boron, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc nutrition for determination of the new Dietary Reference Intakes for the U.S. population. Contributed to the formulation of DRIs and preparation of draft report submitted for NAS review, released in January 2001.

To meet the need for valid yet inexpensive and easy-to-use procedures to routinely assess the relationship between nutrition and behavior, a

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computer software package and associated procedures were developed to automate the administration of standardized neuropsychological tasks designed to assess a variety of cognitive processes (e.g., perception, attention, learning, memory and reasoning) and psychomotor and spatial skills. Initially designed for English speaking adults, tasks and instructions have recently been adapted for use with children, adolescents, elderly, Native Americans, non-English speaking persons, and other groups at risk for mineral deficiency (e.g., athletes). This technology and related methods represent significant contributions to research on the neuropsychological and behavioral effects of nutritional deficiencies and supplementation. Users of this technology are researchers in private industry and in state and federal governments, domestic and foreign. Currently in progress are 7 collaborative research projects using this technology, supported by 4 granting agencies, and involving 7 principal investigators in 3 countries.

Continued updates and enhancements of this technology will ensure its durability. Lack of familiarity with behavioral and computerized testing, the need for careful training of test administrators, and the lack of age- and country-specific norms are current constraints on adoption of this technology.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Presentation to Nonscientific Group:
None.
Media Coverage:
New Dietary Reference Intakes for Micronutrients Released: GF Human Nutrition Research Center Makes Major Contributions. Grand Forks Herald. January 2001.

This research program, including study results, also receives public exposure through frequent coverage by the popular press (local, regional and national newspapers, magazines, radio and television) and industry newsletters and magazines.

9. Scientific Publications:

01. Moulton, P., Apostal, K., Park, R., Cameron, E., Petros, T., Penland, J. The effect of alcohol hangover on performance. Psychonomic Society. 2000. v. 5. Abstract p. A564.

02. Penland, J.G., Lukaski, H.C. Chromium and picolinic acid supplementation affect menstrual symptomatology but not cognitive performance. The FASEB Journal. 2001. v. 15(5). Abstract p. A1089. Presented by J.G. Penland at the Experimental Biology 2001 meeting. Orlando, FL. March-April 2001.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

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National Program(s): 107 100%

Title: BIOCHEMICAL, PHYSIOLOGICAL, AND NUTRITIONAL ROLES OF CERTAIN ULTRATRACE ELEMENTS

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? Y

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Although emerging evidence indicates that certain mineral elements (e.g., arsenic, boron, nickel, silicon and vanadium) often called ultratrace elements can be involved in the prevention or amelioration of disease with nutritional roots, or for the enhancement of health and longevity, insufficient evidence exists to develop credible and data-supported dietary recommendations for these elements to assure health and well-being. Moreover, inadequate knowledge about health benefits of some ultratrace elements that are now only being discovered or defined (e.g., boron in bone and joint health) results in inappropriate and ineffectual reliance on other mineral nutrients (e.g., calcium) to provide these benefits. The lack of appropriate dietary recommendations for ultratrace elements allows charlatans for the purpose of financial gain to inappropriately promote these elements as supplements that can prevent some feared diseases such as cancer, osteoporosis, heart disease, and loss of cognitive function, or can enhance physical appearance. Thus, some of these elements are promoted (e.g., vanadium) in such a way that intakes detrimental to health may be occurring. Finally, the lack of recognition of health benefits provided by some ultratrace elements gives rise to risk assessments and toxicological standards by regulatory agencies that conflict with amounts that are known or predicted to be beneficial to health; this results in unnecessary efforts and expenditure of funds to reduce environmental exposure to amounts that in reality are not harmful, and may actually have health benefits. In summary, defined biochemical functions for ultratrace elements such as arsenic, boron, nickel, silicon and vanadium will establish these elements as essential nutrients, will allow the development of status indicators for the determination of dietary requirements to prevent any decline in health and well-being, and will allow for appropriate risk assessments and toxicological standards.

Animal and human experiments are and will be conducted to define the biochemical and physiological roles of various ultratrace elements including arsenic, boron, nickel, silicon and vanadium. The basic approach is to feed experimental animals and human volunteers diets that contain low, adequate, and/or luxuriant amounts of specific ultratrace elements and other selected nutrients and non-nutrients (postulated to affect the metabolism and utilization of specific ultratrace elements). The response of the animals and humans to the dietary manipulations will be ascertained

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by evaluating appropriate biochemical, physiological and anatomical variables. Biochemical and molecular biology methods will be used to define the specific essential role(s) of various ultratrace elements.

2. How serious is the problem? Why does it matter?

Dietary factors are associated with 5 of the 10 leading causes of death and with numerous chronic disorders; these include coronary heart disease, certain types of cancer, stroke, atherosclerosis, hypertension, osteoporosis and arthritis. Among those diseases that are linked strongly to diet, the cost of treatment and care in the United States exceeds $200 billion per year. Recognition that nutrition is important in health promotion and disease prevention has spawned a plethora of "health-enhancing foods" and supplements, now often called "functional foods" or "nutraceuticals" that represent an exploding market in the United States which exceeds $29 billion per year. Many of the health claims for these functional foods and nutraceuticals, however, have not been substantiated by basic research and feeding trials. Many of the health claims include the use of ultratrace elements because of some promising physiological or clinical finding (most often in an animal model or a special human situation) that has been extrapolated to their intakes having an influence on the susceptibility to, or severity of, one or more chronic diseases. Thus, there is a need to establish which foods, and their amounts, that will provide appropriate quantities of specific ultratrace elements of practical importance for the promotion of health and disease prevention or alleviation. Additionally, there is a need to determine safe intakes of specific ultratrace elements so that the setting of reasonable toxicological standards can be accomplished. Fulfilling these needs should result in policies and programs that will result in a healthier population, decrease the burden of chronic disease, enhance the quality of life, and diminish health care and environmental exposure protection expenditures.

3. How does it relate to the National Program(s) and National Program Component(s)?

The research program relates to the National Program 107, Human Nutrition, and emphasizes the Program Component Performance Goal 3.1.1 - Human Nutrition Requirements. The challenge of this component is to identify essential nutrients, determine their effects on reproduction, development, function and longevity, and to provide information that will be used to develop standards to optimize human health, well-being, and genetic potential throughout the life cycle. All priority objectives, especially mechanism of action, biomarkers, function and performance, and nutrient interactions apply to the research program. Outcomes of the research will be knowledge that will facilitate the detection and prevention of biochemical, structural, physiological and psychological dysfunctions caused by the deficiency or imbalance of specific ultratrace

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elements, and will define requirements and safe intakes of specific ultratrace elements for health and well-being throughout the life cycle.

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishment during FY 2001:
The physiological function of nickel has not been defined but recent findings have suggested that it affects the cyclic GMP signal transduction system, and thus would affect kidney function including blood pressure control. At the Grand Forks Human Nutrition Research Center, male weanling rats were fed for nine weeks nickel-deficient or nickel-adequate diets with or without excessive sodium chloride. Nickel deprivation induced high blood pressure, and increased blood and albumin in the urine, and exacerbated the blood pressure increase and kidney changes induced by sodium chloride. The findings suggest that nickel has essential function involving cyclic GMP signal transduction which is of nutritional importance because it may be a factor that contributes to the incidence of high blood pressure including that provoked by high sodium chloride intake.

B. Other Significant Accomplishment(s):
Because of the existence of evidence suggesting that nickel affects the cyclic GMP signal transduction system, it was hypothesized that nickel deprivation would affect sperm motility which is influenced by this system. At the Grand Forks Human Nutrition Research Center, male weanling rats were fed for nine weeks nickel-deficient or nickel-adequate diets with or without L-NAME (a nitric oxide synthase inhibitor; nitric oxide affects the cyclic GMP signal transduction system) in their drinking water the last four weeks. Nickel deprivation decreased sperm density and motility; sperm density was lowest in nickel-deprived rats fed L-NAME. The findings suggest that nickel is an essential nutrient that is physiologically important in functions involving the cyclic GMP signal transduction system including that which could affect ability to produce offspring.
Selenium supplementation is recognized as being able to decrease the risk for certain types of cancer (e.g., prostrate cancer) and heart disease (e.g., Keshan disease). At the Grand Forks Human Nutrition Research Center, a study was performed with rats that determined the effect of dietary selenium on homocysteine (an independent risk factor for atherosclerotic disease) and DNA methylation (hypomethylation of DNA is associated with increased cancer susceptibility). Low dietary selenium decreased plasma and tissue concentrations of homocysteine and the activity of liver betaine homocysteine methyltransferase, increased liver glutathione and resulted in hypomethylation of liver DNA. These findings suggest that enzymes in methionine metabolism are affected by selenium deprivation and provide evidence that selenium may influence cardiovascular health and cancer risk through an effect on homocysteine/methionine metabolism.

C. Signficiant Accomplishments/Activities that Support Special Target

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Populations:
None.

D. Progress report.
No report needed.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Normal or nutritional dietary intakes of boron were found to help control the normal inflammatory process and therefore reduce the development of inflammatory disease. In arthritic animal model systems, physiological amounts of dietary boron increased concentrations of natural killer cells, attenuated the rate of paw swelling, and reduced the incidence of an inflammatory state. These findings suggest that physiological amounts of boron reduce the risk for inflammatory disease by helping hold in check a system that is constantly poised to attack, a balance that permits pathogen elimination but avoids autoimmunity. The national cost of rheumatoid arthritis, an inflammatory disease, is similar to that estimated for heart disease and stroke. The finding that boron controls the degree of severity of a form of experimental rheumatoid arthritis suggests that dietary recommendations for boron could have an impact on the occurrence and severity of this disease.

Dietary boron, fed in nutritional amounts, changed the maturation rate of the developing spinal column in an animal nutrition-pregnancy model. This is the first report indicating that boron has a role in the earliest stages of bone formation in mammals. This finding, along with the observation that boron interacts with erythritiol (a boron-binding substance) to affect fetal absorptions in the pregnancy model, suggest that boron may be important in embryo development in humans and supports the concept that boron is an essential nutrient for humans.

Hypomethylation of DNA is associated with an increased incidence of certain forms of cancer. When compared to cells cultured in media containing small amounts of arsenic, cells cultured in media containing negligible amounts of arsenic hypomethylated DNA. This finding demonstrates that there are beneficial amounts of arsenic that can decrease the risk to certain forms of cancer, in addition to toxicological amounts that can increase that risk, and that toxicological standards need to consider that both too little and too much arsenic can have detrimental consequences.

Vanadium was found to be a nutritionally important element involved in thyroid hormone function and glucose metabolism. The amount needed for this involvement was extremely small, and thus indicated that supranutritional amounts, similar to those provided by supplements available over-the-counter to the public, could induce changes that could be construed as not beneficial. This suggests that some over-the-counter supplements and formulas sold as anabolic or anti-diabetic agents could be detrimental to

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health.

Vanadium status affected the response of rats to N-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide (an important reactive oxygen species) formation, and oxidative metabolism status indicators. The findings suggest that vanadium has a biological role involving a reactive oxygen species and supports the concept that vanadium is an essential nutrient but needed in very low amounts.

Arsenic deprivation was shown to be important in methionine metabolism apparently by affecting methionine recycling. Two metabolites of methionine were altered by arsenic deprivation in rats; liver S-adenosylhomocysteine (SAH) was increased while S-adenosylmethionine (SAM) was decreased which resulted in a decreased ration of SAM to SAH. A low SAM/SAH ratio has been associated with an increased risk for certain types of cancer; thus, although high arsenic apparently can increase risk to certain types of cancer, the findings indicate that too low of intakes of arsenic can also increase the risk to certain types of cancer. This possibility should be considered by groups setting toxicological standards for arsenic because standards set too low could result in unnecessary expenditure of funds to reduce oral intakes to amounts that could actually have unfavorable health effects.

Arsenic was found to be a nutritionally beneficial ultratrace element whose effects are modified by stressors of sulfur amino acid metabolism (e.g., methionine). Among the enzymes involved in this metabolism whose activity was altered by arsenic when stressors were present was the liver enzyme betaine-homocysteine methyltransferase. This indicates that arsenic has a nutritional role involving biological methylation and support the concept that arsenic is an essential nutrient.

An interaction between arsenic and copper showed that high dietary arsenic can exacerbate signs of copper deficiency. Extremely high arsenic (compared to normal nutritional intakes) had adverse effects on ceruloplasmin, heart size and the concentration of mineral elements in liver and kidney in rats fed low dietary copper. Thus, pathological conditions such as cardiovascular disease attributed to arsenic toxicity may in reality be caused by copper deficiency. Increasing copper intakes may be the most efficient and cost-effective mechanism of decreasing the incidence of heart disease associated with elevated amounts of arsenic in drinking water.

By the use of the isotope nickel-63, it was found that 2.5% of nickel ingested by rats is absorbed and very little is retained. A model suggesting that nickel is homeostatically controlled by absorption and retention processes was developed. In this model, nickel is metabolized by at least three different mechanisms. The model and findings provide further evidence that nickel is an essential element for higher animals and humans, and thus could be of nutritional importance.

Novel mathematical approaches (logistic regression, discriminant analysis) were developed that can be used to derive more objective-based dietary guidance and can be easily adapted to new information as it becomes

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available. Because the models account for interactive effects (synergistic or antagonistic), they can be tailored to a specific group of individuals. The mathematical approaches also have given an improved method for assessing the status of specific nutrients which uses mathematically combined parameters rather than individual parameters; the combined parameters give a better correlation to the status of the nutrient in question. More accurately determining the need for mineral nutrients will promote the intakes necessary for optimal health and well-being.

6. What do you expect to accomplish, year by year, over the next 3 years?

Terminated.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Information about the nutritional or beneficial aspects of ultratrace elements as it becomes available is routinely transferred to a variety of customers. The customers include risk assessments groups through direct contact or organized meetings and workshops; the public through web pages of professional organizations and the Grand Forks Human Nutrition Research Center, and via popular media; and other scientists through presentations at national and international meetings and professional publications.

Information was transferred to the public through an article in the local newspaper (Grand Forks Herald) which also was placed on the Grand Forks Human Nutrition Research Center Home Page; this article was "Hoodwinking Health Articles" by Forrest H. Nielsen.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

Articles in the popular media often mention boron as a nutrient important for bone and joint health; this is based on findings from research done in this CWU. No attempt is made to keep a record of these articles.

9. Scientific Publications:

01. Coughlin, J.R., Nielsen, F.H. Advances in boron essentiality research: symposium summary. New Aspects of Trace Element Research. Abdulla, M. Bost, S. Gamon, P. Arnaud, G. Chazot, editors. Smith-Gordon, London. 1999. p. 33-41.

02. Uthus, E.O. High dietary arsenic exacerbates copper deprivation in rats. Journal of Trace Elements in Experimental Medicine. 2001. v. 14. p. 43-45.

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Publications: (Continued)

03. Davis, C.D., Uthus, E.O., Finley, J.W. Dietary selenium and arsenic affect DNA methylation in vitro in caco-2 and in vivo in rat liver and colon. Journal of Nutrition. 2000. v. 130. p. 2903-2909.

04. Armstrong, T.A., Spears, J.W., Crenshaw, T.D., Nielsen, F.H. Boron supplementation of a semipurified diet for weanling pigs improves feed efficiency and bone strength characteristics and alters plasma lipid metabolites. Journal of Nutrition. 2000. v. 130. p. 2575-2581.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

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Accession: 0400356 Year: 01 Project Number: 5450-51530-003-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.3.1.1 25%
National Program(s): 107 100%

Title: MINERAL ELEMENTS, PHYSIOLOGICAL FUNCTION & PERFORMANCE AND BODY COMPOSITION

Period Covered From: 10/00 To: 09/01

Would you like to terminate this Project? Y

Will this project terminate within the first 2 months of FY2002? N

Progress and Outcomes:
1. What major problem or issue is being resolved and how are you resolving it?

Recommendations for the dietary intake of mineral elements, with an emphasis on copper, chromium, iron, magnesium, and zinc, based on the promotion of health and optimal biological function are generally lacking. Studies designed to examine the effects of graded dietary intakes of mineral elements on physiological function are needed to ascertain appropriate amounts of mineral elements in the diet to maintain health and to facilitate the attainment of genetic potential of biological functions. One consideration in delineating appropriate dietary mineral intakes is assessment of food-borne factors that affect the absorption and utilization of dietary minerals. Also, the use of environmental stressors (i.e., controlled exercise and cold/hot temperatures) is another factor used to determine mineral element needs of physically active people.

Iron deficiency is the single most prevalent nutritional deficiency in the world. Attempts to fortify food products have been only partially successful in ameliorating this pervasive nutritional problem among women. In parallel with the incidence of iron deficiency, there has been an increase of polyunsaturated fat intake. Studies indicate that the type of dietary fat affects iron absorption and utilization, specifically non-heme iron. Polyunsaturated fat reduces and saturated fat, specifically stearic acid, promotes iron utilization. Because stearic acid is neutral to serum cholesterol and lipoprotein cholesterol concentrations, it offers practical benefits in ameliorating iron deficiency in animals and humans.

Routine assessment of human body composition is hampered by the lack of sensitive and specific methods to measure bone mass and quality, fat and muscle in national nutritional surveys and repeatedly in response to medical and nutritional interventions.

Studies are conducted in animals and humans. Graded intakes of dietary zinc and copper are fed and physiological functions are monitored to delineate intake amounts that affect physiological function with an emphasis on energy utilization, cardiorespiratory function, work performance, and heat production. Studies of human volunteers fed whole food diets low in chromium and supplemented with specific chromium compounds and other compounds hypothesized to influence the absorption and utilization of chromium and other minerals are conducted. Other studies are undertaken in which animals are fed a diet low in iron, then given diets containing varied amounts of iron (low and adequate) and different types

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(saturated and polyunsaturated) of dietary fat. Changes in iron status and hematology, as well as changes in bone mineral content, are determined.

Studies are undertaken in animals and humans to develop and validate methods for use in assessing nutritional status and effects of nutritional intervention on bone and soft tissue composition.

2. How serious is the problem? Why does it matter?

There is considerable debate regarding the amount of dietary copper, chromium, magnesium, and zinc required for health maintenance and optimal biological function. Previous approaches focused on relatively insensitive measures of nutritional adequacy (chemical balance). By relating dietary mineral intakes to measurements of biological function, such as energy utilization, heart rate and blood pressure, work production, heat generation, and glucose and lipid metabolism, suggestions for dietary mineral intakes are made in reference to quality and quantity of life. Much of this research is requested by physically active individuals who seek to optimize physiological function without the use of dietary supplements and health managers who seek to minimize health care costs of the American public.

Development and validation of sensitive and specific methods to assess human body composition remain fundamental needs for nutritional assessment of healthy persons of all ages and backgrounds as well as individuals with chronic disease and medical intervention. Moreover, these methods are required for establishment of national distributions or norms for body fat and muscle for use as references in nutritional interventions to promote health.

3. How does it relate to the National Program(s) and National Program Component(s)?

This work relates to National Program 107, Human Nutrition(100%). The specific Program Components include Nutrient Requirements with the objectives of defining Biomarkers of Marginal or Borderline Deficiencies, Mechanisms of Action of Mineral Elements, Effects of Environmental and Lifestyle Factors, and Achievement of Optimal Function and Performance.

This research will acquire information about the effects of graded trace element deficiencies, emphasizing copper, chromium, iron, magnesium, and zinc, on biochemical measurements of mineral nutritional status and physiological functions. This information will facilitate the detection of marginal mineral deficiencies and define dietary requirements of these mineral elements for the development and maintenance of health and optimal function throughout the life cycle. The research will provide needed information that can be used to assess the risk of chronic diseases and impairments in subtle physiological functions that arise from mild and moderate mineral element deficiencies. In addition, new methods for routine assessment of human body composition will be developed and validated for

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non invasive assessment of human nutritional status, particularly conditions that exhibit weight change.

4. What were the most significant accomplishments this past year?

A. Single Most Significant Accomplishments during FY 2001:
Although altered zinc status is observed in certain groups of physically active persons, knowledge of the interaction between zinc intake and physical training on biochemical measures of zinc status is scanty. Male weanling rats were fed diets containing 3 or 18 mg zinc and matched by weight into groups that either remained sedentary or trained to run on a treadmill for 8 weeks. Exercise training increased plasma zinc, bone zinc and activities of red blood cell carbonic anhydrase activity except in the animals fed a diet low in zinc. Impact: These findings show that exercise promotes functional adaptation in zinc metabolism favoring increased activity of zinc-containing enzymes in response to increased cardiorespiratory demands.

B. Other Significant Accomplishment:
Methods for assessment of human body composition, with an emphasis on muscle mass, in the field are lacking. In conjunction with physicists at Northeastern University, we refined the standard single-frequency bioelectrical impedance method for routine measurement of thigh muscle mass in adults of varying age and body composition. By using a parallel rather than a series electrical model and incorporating measures of subcutaneous fat into a model, we found that electrical estimates of muscle volume, and hence mass, corresponded with reference determinations of muscle. Impact: The refined method provides a simple and accurate technique for routine determination of muscle mass in humans.

C. Significant Accomplishments/Activities that Support Special Target Populations:
None.

D. Progress Report.
None.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Chromium is a mineral element for which a recommended dietary allowance (RDA) has not been established. Healthy premenopausal women were randomized into treatment groups receiving a placebo, chromium picolinate (200 micrograms chromium)and picolinic acid and fed a whole-food diet containing an average of 35 micrograms of chromium for 12 weeks. Compared to placebo, neither chromium nor picolinic acid supplementation had any effect on body

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composition or nutritional status indicators. These findings indicate that chromium supplementation does not promote weight loss, favorable changes in body composition or impair nutritional status of other minerals; apparently 35 micrograms of chromium meets the needs of premenopausal women.

Epidemiological surveys suggesting that the prevalence of obesity is increasing in the United States used only crude measurements of standing height and body weight. Thus, more valid methods such as bioelectrical impedance are needed to measure body composition of a nationally representative sample of American adults and children to determine the validity of the suggestion. Investigators from the USDA, ARS Grand Forks Human Nutrition Research Center, St. Luke's-Roosevelt Hospital, Wright State University, and University of Chicago, with funding from the National Institutes of Health and the National Center for Health Statistics, developed and validated models to predict body composition based on measurements of bioelectrical impedance, then used impedance data from the Third National Health and Nutrition Examination Survey (NHANES III) in these models to determine the body composition of nationally representative samples of American children and adults. This study produced the first estimates of fat-free mass and body fat for non-Hispanic white, non-Hispanic black and Mexican Americans aged 12-80 years. These data provide an interim distribution of body composition parameters among three racial groups that will be useful in assessing future trends in fatness, a predictor of chronic disease, and fat-free mass, a key marker of sarcopenia, in the US population.

Simple, non-invasive methods, which are accurate and reliably determine changes, to assess human nutritional status, particularly muscle mass, are lacking. We adapted the tetrapolar bioelectrical impedance method, that was developed at the Grand Forks Human Nutrition Research Center, for the assessment of muscle mass in the upper legs (thighs) of overweight women who participated in a controlled weight loss program. Results showed that the bioimpedance determinations of muscle were similar to the reference measurements made with dual x-ray absorptiometry. Impact: These initial findings indicate that this new method is a valid and accurate method for routine determination of muscle mass in humans; this impedance approach also was a better indicator of muscle mass than the currently used measures of thigh circumference to estimate muscle mass.

Assessment of zinc nutritional status is complicated by the limited availability of blood biochemical markers that clearly distinguish between adequate and deficient zinc status and differentiate between degrees of zinc status, marginal and severe deficiency states. We evaluated the response of a zinc-containing enzyme in plasma, extracellular superoxide dismutase, in a series of studies of rats fed different amounts of dietary zinc and zinc-deficient rats supplemented with zinc. We found that the activity of this enzyme was more responsive to differences in dietary zinc, particularly in the rehabilitation of the zinc-deficient rats, than the currently used biochemical indicators of zinc status, including plasma zinc. Impact: These findings suggest that extracellular zinc superoxide

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dismutase activity is a more specific marker of zinc status than currently available indicators of zinc status, such as plasma zinc concentration, and that it may be useful in assessing human zinc nutritional status among individuals in national nutritional surveys.

Restricted dietary zinc adversely affected energy utilization during exercise in young men. As compared to a dietary zinc intake of 18 mg/d, 3 mg zinc daily was associated with significant alterations in energy production and respiratory function during progressive peak exercise on a cycle ergometer. The mechanism of this impairment was a significant decrease in red blood cell carbonic anhydrase activity with restricted dietary zinc. Carbonic anhydrase is a zinc-containing enzyme with the specific function of transporting carbon dioxide from cells to the lungs for excretion. Zinc deficiency was confirmed with a significant loss of zinc, negative zinc balance, and decreased serum zinc concentration when dietary zinc was restricted. Impact: These findings provide the first evidence of impaired physiological function when dietary zinc is fed in an amount similar to that consumed by some physically active individuals. Also, the finding of decreased carbonic anhydrase activity in the red blood cell in response to low dietary zinc suggests that the activity of this zinc-containing enzyme may be a new blood biochemical marker for assessment of human zinc nutritional status.

Blood cells are not an appropriate tissue for determination of chromium nutritional status. A pilot study revealed that the chromium concentrations of populations of white blood cells and platelets were too low (i.e., similar to background) to be useful as diagnostic measures of human chromium status. Although efforts to reduce background contamination were successful, the very low concentrations of chromium in these cells were difficult to measure accurately. Impact: The static measurement of cellular chromium is inadequate as a routine determination of human chromium nutritional status.

Restriction of dietary copper resulted in altered temperature regulatory function of rats acutely exposed to cold air. Copper deficiency was associated with an increased rate of loss of body temperature, decreased enzymatic conversion of the active form of thyroid hormone, triiodothyronine, from thyroxine, and decreased activity of the rate limiting enzyme dopamine beta hydroxylase, a copper-containing protein, needed for increased production of norepinephrine, the key regulator of heat production. A key finding was the identification of decreased transcription and translation of uncoupling protein that is required for heat production and thermogenesis in brown adipose tissue. The lack of induction of uncoupling protein formation is caused by a depressed expression of the genetic message for a specific heat shock protein, HSP 70, in copper deficiency. Importantly, the adverse effects of copper deprivation are ameliorated with copper repletion within three days of copper supplementation. Impact: These findings provide important information explaining the mechanism through which copper regulates energy metabolism, and explain why humans with an inborn inability to absorb

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copper suffer from hypothermia despite adequate hemoglobin concentration.

Supplementation of men with chromium picolinate while participating in controlled resistance training failed to demonstrate increased strength gain, facilitate loss of body fat and enhance muscle mass accretion. Impact: The results of this controlled study served as the basis for the Federal Trade Commission to rule that claims of propitious effects of chromium picolinate supplementation were without scientific basis. Similarly, the U.S. Pharmacopeia has concluded that chromium picolinate does not promote weight loss, facilitate body fat loss or promote muscle mass gain.

Consumption of dietary magnesium in amounts generally consumed by U.S. women resulted in alterations in energy production during submaximal exercise. Postmenopausal women fed 150 mg of magnesium daily demonstrated an increased oxygen consumption and elevated heart rates during submaximal exercise on a cycle ergometer as compared to a diet providing 350 mg magnesium daily, the recommended dietary intake. Magnesium deficiency was documented with increased losses of magnesium and altered blood ionized magnesium, that is consistent with increased mobilization of magnesium from bone, and decreased skeletal muscle magnesium. Impact: These findings provide the first evidence of diet-induced magnesium deficiency in otherwise healthy adults, and demonstrate that dietary magnesium intake consistent with amounts generally consumed by a majority of U.S. women are inadequate to support moderate intensity physical activities of daily life. Because significant physiological impairments were found at magnesium intakes consistent with national estimates of usual intake, there is a need for increased public health education to bolster dietary magnesium intakes to maintain physiological function and health.

Stearic acid promotes non-heme iron absorption and utilization in iron-deficient animals. Studies in rats and canines repeatedly showed that stearic acid enhanced the uptake and transfer of non-heme iron from the intestinal mucosa and increased red blood cell volume and hemoglobin concentration. This beneficial effect occurred at high (30%) and moderate (20%) intakes of stearic acid. Furthermore, the enhancement of iron metabolism with stearic acid did not adversely impact calcium or magnesium status. Impact: Stearic acid may be the uncharacterized component of meat, the "meat factor", that promotes non-heme iron utilization. Although saturated fatty acids generally increase atherogenic risk by increasing serum cholesterol and low density lipoprotein cholesterol concentrations, stearic acid has no adverse effects on cholesterol or lipoproteins. Use of stearic acid in recipes containing non-meat foods may reduce the incidence of iron deficiency anemia in humans.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY 2001: Develop, validate and implement a new technical method for the use of tetrapolar bioelectrical impedance analysis for the assessment of regional muscle mass in humans. A model will be developed in weight-stable

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adults for determination of thigh muscle mass by using magnetic resonance imaging as a reference method. This model will be evaluated in adults participating in resistance training to increase muscle mass. This study will determine the advantages of specific impedance instruments (i.e., single or multiple frequency) in the predictive accuracy and sensitivity of muscle and fat determinations. If this approach is successful, a practical method will be available for generalized use in the routine assessment of human nutritional status in national surveys and hospital use with patients gaining or losing muscle and fat mass.

FY 2001: Determine the individual and combined effects of graded dietary zinc and copper on activities of superoxide dismutase enzymes as markers of zinc and copper status relative to traditional biochemical markers of zinc and copper nutriture of rats. Incorporate measures of extracelluar superoxide dismutase protein to assess the specific activity of this enzyme as a marker of zinc status and evaluate if expression of message of this protein is affected by dietary minerals. Findings will provide a basis for planning future human studies to delineate mineral requirement to control oxidative damage during physical training.

FY 2001: Ascertain the effects of graded dietary copper on resting energy metabolism and thermogenesis in the cold in rats. Test the hypothesis that copper restriction impairs the induction and expression of uncoupling proteins. Determine the effects of copper depletion independently of anemia on the metabolic perturbation in energy metabolism of copper-deficient rats. This study will identify new metabolic role(s) of copper in regulating energy expenditure.

FY 2001: Delineate the effects of dietary zinc and exercise on the induction and expression of zinc-containing enzymes involved in energy production in rodents. By using graded dietary zinc and different types of exercise training (endurance and resistance), this study will determine if limiting zinc intake has adverse effects on ability to improve physical performance by limiting the production and activity of specific zinc-containing enzymes. Findings will provide a practical basis for assessing zinc requirements of physically active persons.

FY 2002: Determine the effect of type and amount of fatty acids on the transport and uptake of iron and other mineral elements by intestinal cells. Determine the induction and expression of cellular transport proteins of Caco-2 cells in vitro to elucidate the molecular mechanisms of dietary fat on trace element absorption. Results will define the molecular mechanisms of action of different fatty acids on iron uptake and transport and will compliment findings from studies of animals.

FY 2002: Plan and initiate a health and nutrition survey of Native American tribes in North Dakota to determine relationships among mineral nutritional status and health with an emphasis on obesity, diabetes and cardiovascular disease. Results will provide a basis for planning future mineral supplementation trials to test the hypothesis that remediation of mineral deficiency will decrease incidence of some chronic diseases.

FY 2002: Plan and initiate studies in humans to determine the effects of

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altered fatty acid composition of oilseeds and meat on circulating lipids and lipoproteins and hence risk of chronic disease. Results will be used to support large trials of fatty acid modification in grains and meat to promote human health and enhance value added nature of certain agricultural products.

FY 2003: Determine the effects of increased zinc intake, either as food fortification or as supplement, on growth and body composition of adolescents with varying zinc status. Findings will be used to support food fortification strategies to optimize growth and function in a group that has an increased need for zinc because adolescence is a period of rapid growth and development.

FY 2003: Design and implement a study of older adults to discern the impact of antihypertensive medications on zinc and magnesium status and assess the impact of supplemental zinc and magnesium, either as supplements or food fortification, on muscle function and body cell mass.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Information about the basic and applied aspects of mineral nutrition as it becomes available is routinely transferred to a variety of customers. The customers include other federal agencies and national and international sport groups through direct contact or organized meetings and conferences; the public through web pages of professional organizations and the Grand Forks Human Nutrition Research Center, and via popular media; and other scientists through presentations at national and international meetings and professional publications. Results from studies are published in peer-reviewed scientific journals. Upon publication, the methodology, data, and interpretations of the data are immediately available to scientists and other interested parties. In addition, experimental results and educational information pertinent to public interests are made available through the local newspaper, The Grand Forks Herald, and statewide through the Interactive Video Network of the North Dakota State University Service Continuing Education Program.

8. List your most important publicatons in the popular press (no abstracts) and presentations to nonscientific organizations and articles written about your work (NOTE: this does not replace your peer-reviewed publications which are listed below)

American Dietetics Association, Food and Nutrition Conference, Office of Dietary Supplements Symposium on Is There a Role for Dietary Supplements in the Management of Diabetes? Lessons from Clinical Trials. October 16, 2000, Denver, CO. Lecture - Lessons from Micronutrient Studies in Patients with Glucose Intolerance and Diabetes Mellitus: Chromium and Vanadium.

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Brigham and Women's Hospital, October 26, 2000, Boston, MA. Lecture - Clinical Applications of Regional Bioelectrical Impedance Analysis to Assess Regional Muscle Mass and Nutritional Status of Hospitalized Patients.

University of North Dakota School of Medicine and Health Sciences, Department of Biochemistry, November 28, 2000, Grand Forks, ND. Lecture - Diet, Nutritional Supplements and Physical Activity - What Physicians Should Know.

University of Colorado Health Sciences Center, Center for Human Nutrition, January 29, 2001, Denver, CO. Lecture - Effects of Dietary Magnesium and Zinc Restriction on Metabolic Responses to Exercise in Humans.

University of North Dakota School of Medicine and Health Sciences, February 21, 2001, Grand Forks, ND. Lecture - Teen Nutrition and Childhood Obesity.

9. Scientific Publications:

01. Lukaski, H.C. Bioimpedance. Pierson, R.N., editor. Springer-Verlag, New York, NY. Quality of the Body Cell Mass: Body Composition in the Third Millenium. 2000. p. 76-88.

Approved: HENRY C LUKASKI Date: 08/01
Title: ACTING CENTER DIRECTOR

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